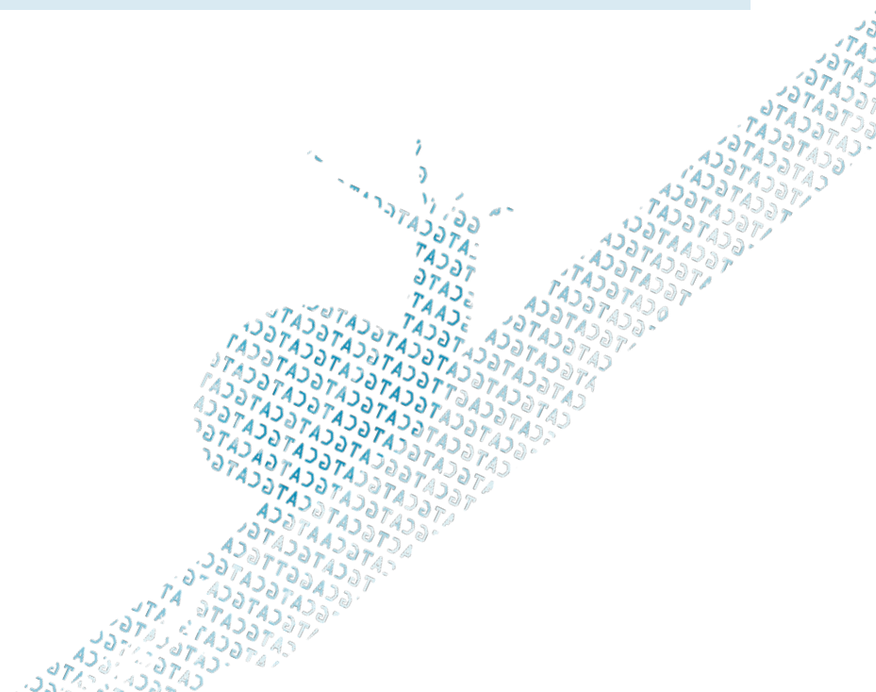
Genetic Diversity Centre Zurich

Genetic Diversity: Analysis

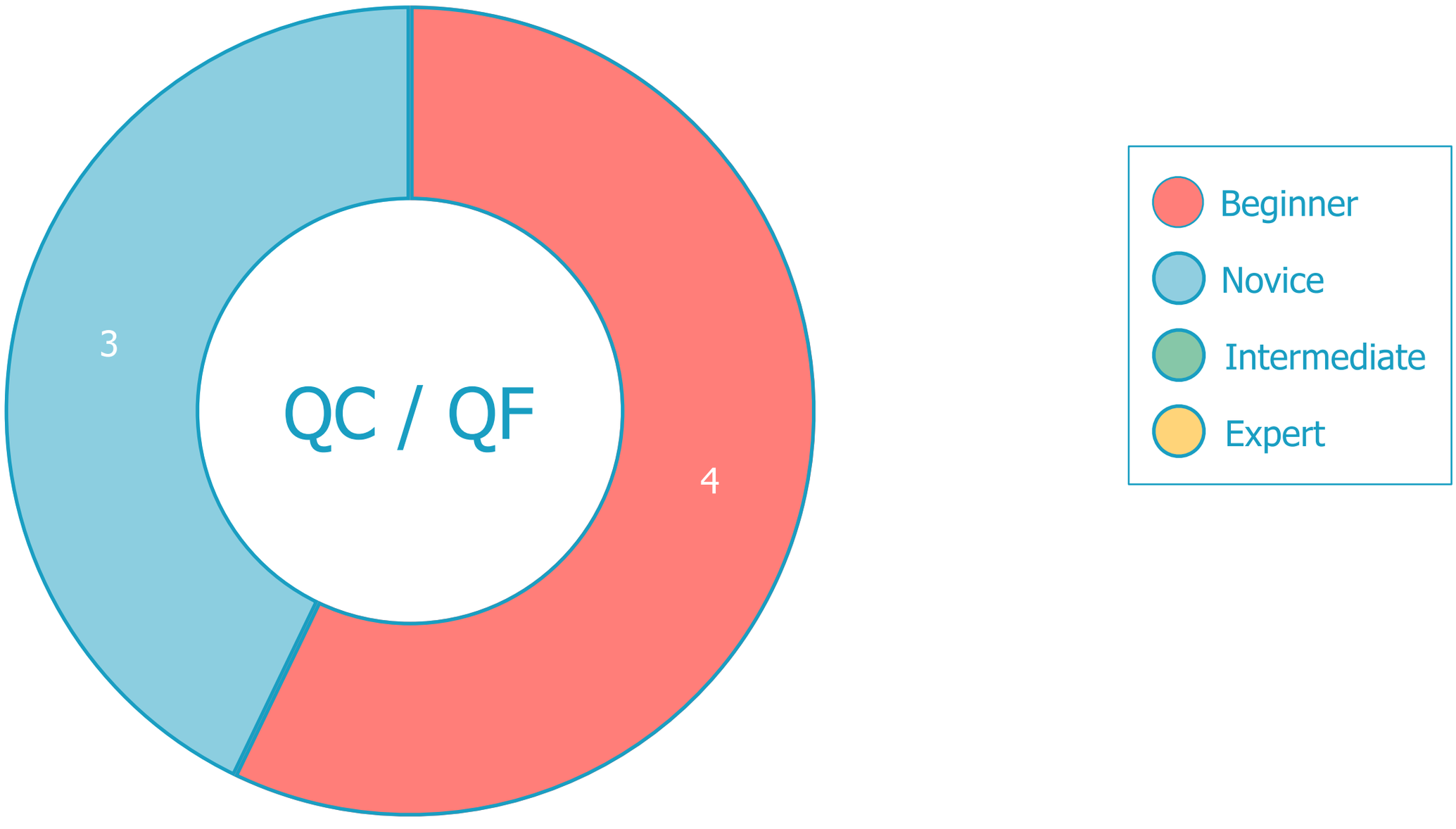
Sequence Quality Control

Friday, June 20, 2025



ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

MPS ▷ Quality Control



Genetic Diversity Centre Zurich

1 **Sample** Quality Control

2 **Library** Quality Control

3 **Run** Quality Control

4 **Sequencing** Quality Control

5 **Data** Quality Control (e.g. bias, outlier)

Genetic Diversity Centre Zurich

1 **Sample** Quality Control

2 **Library** Quality Control

3 **Run** Quality Control

4 **Sequencing** Quality Control

5 **Data** Quality Control

Run QC

MPS ▷ Quality Control

Sequencing Analysis Viewer

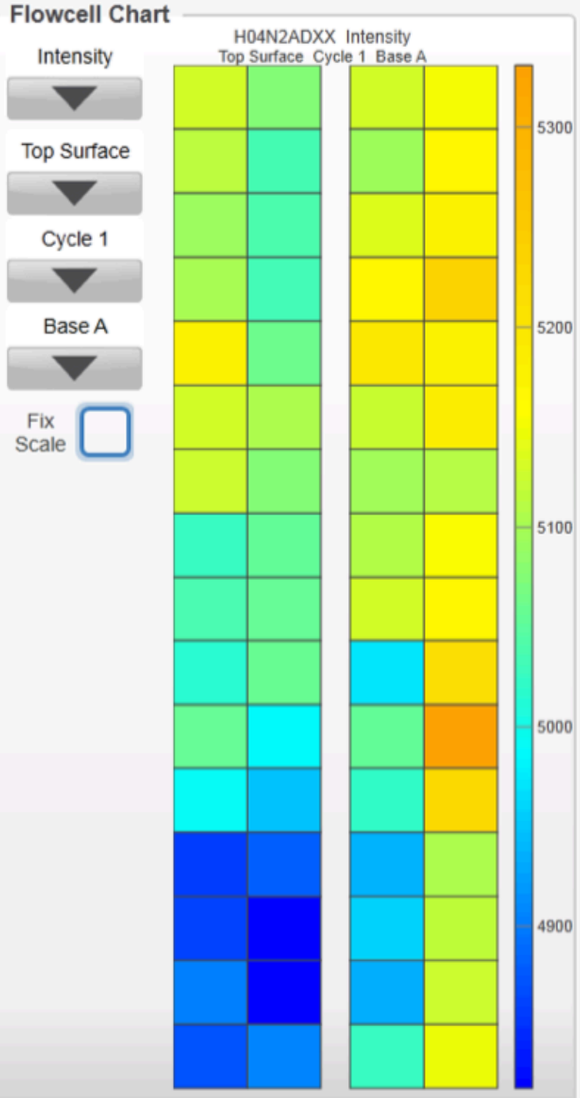
Run Folder: 121019_SN700304R_0494_AH04N2ADXX Browse Refresh

Analysis | Imaging | Summary | Tile Status | TruSeq Controls

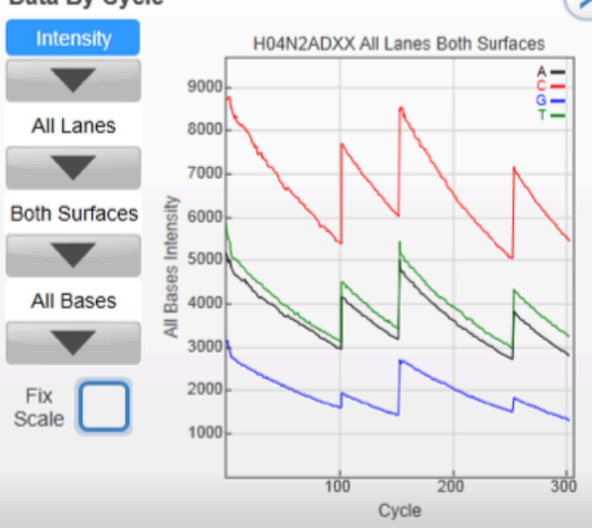
Status

Extracted: 302 Called: 302 Scored: 302

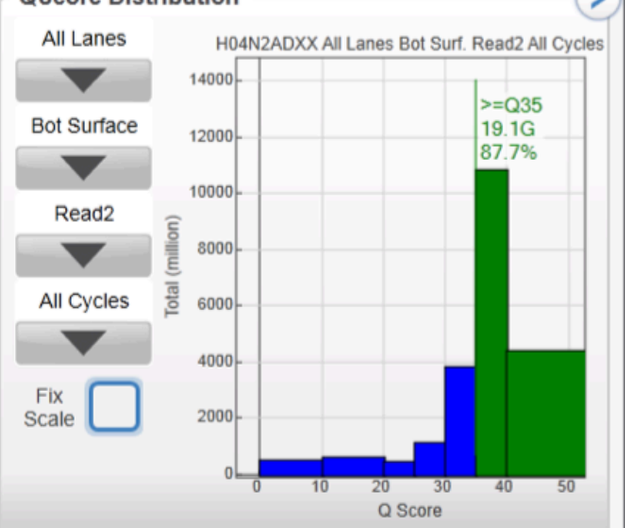
Flowcell Chart



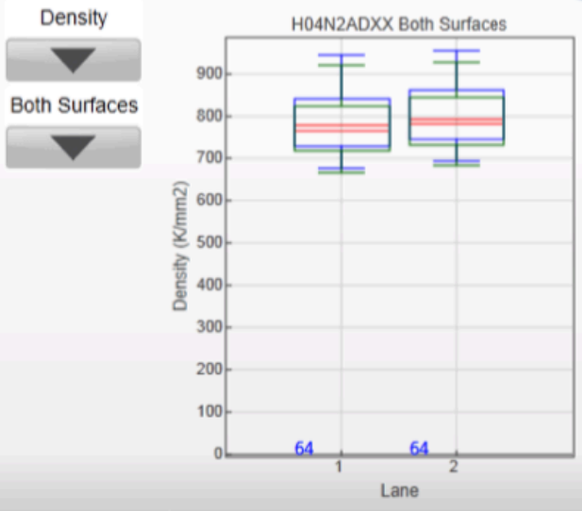
Data By Cycle



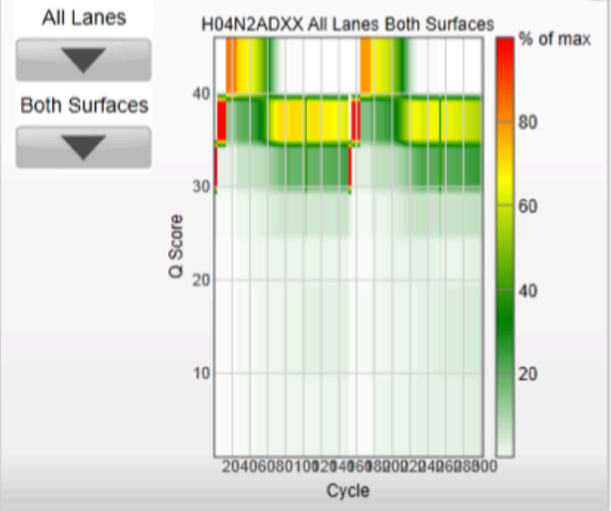
QScore Distribution



Data By Lane



Qscore Heatmap



Illumina Sequencing Analysis Viewer 1.8.37 - 151204_M03930_0010_000000000-AK6GL

Sequencing Analysis Viewer

Run Folder: D:\Illumina\MiSeqAnalysis\151204_M03930_0010_000000000-AK6GL Browse Refresh

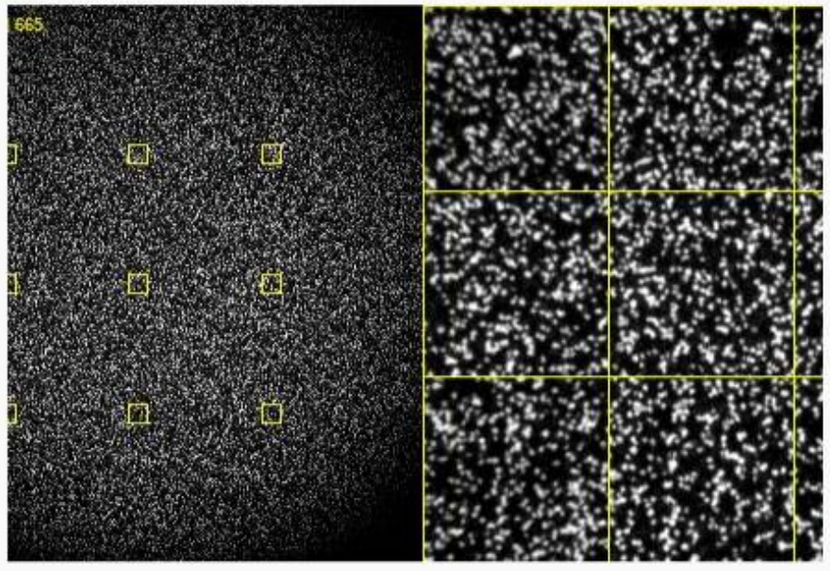
Analysis | Imaging | Summary | Tile Status | TruSeq Controls | Indexing

Cycle All | Lane All | Surface Both | Swath All

A C G T

Index	Lane	Tile	Section	Cycle	Surface	Swath	Time	P90 A
1	1	1101	1	1	Top	1	12/04/201...	363
2	1	1101	1	2	Top	1	12/04/201...	369
3	1	1101	1	3	Top	1	12/04/201...	378
4	1	1101	1	4	Top	1	12/04/201...	382
5	1	1101	1	5	Top	1	12/04/201...	375
6	1	1101	1	6	Top	1	12/04/201...	381
7	1	1101	1	7	Top	1	12/04/201...	392
8	1	1101	1	8	Top	1	12/04/201...	381
9	1	1101	1	9	Top	1	12/04/201...	406
10	1	1101	1	10	Top	1	12/04/201...	396
11	1	1101	1	11	Top	1	12/04/201...	404
12	1	1101	1	12	Top	1	12/04/201...	399
13	1	1101	1	13	Top	1	12/04/201...	400
14	1	1101	1	14	Top	1	12/04/201...	402
15	1	1101	1	15	Top	1	12/04/201...	412
16	1	1101	1	16	Top	1	12/04/201...	426
17	1	1101	1	17	Top	1	12/04/201...	423

Rows=14504 Disp=14504 Sel=1 Filter



Cluster Density: 1017 K/mm2 (optimal 865-965 k/mm^2)
Reads Total: 27.69 M (goal 30 M)
Reads PF: 21.60 M (78%)
PhiX Conc: 2.03 % (loaded 2%)
%>=Q30: Total 63.06% (should be at least 70%)

- The **density** of clusters for each tile (in thousands per mm^2) and the number of **clusters** for each tile (in millions).
- Total **yield** is the number of bases generated in the run.
- The calculated **error rate**, as determined by a spiked in PhiX control sample if available and it refers to the percentage of bases called incorrectly at any one cycle.
- The total fraction of passing filter reads (**PF**) assigned to an index.
- **% Q-score** >= Q30 (percentage of bases that have a Q-score above or equal to 30; Q30 is a probability of incorrect base calling of 1 in 1000).
- The **signal to noise ratio** is calculated as mean called intensity divided by standard deviation of non-called intensities. Not calculated for NextSeq two-channel sequencing or HiSeq X.
- The percentage of molecules in a cluster for which sequencing falls behind (**phasing**) or jumps ahead (**prephasing**) the current cycle within a read.

Sequence QC

Data Format(s)

- Fasta
- Fastq (Fasta with Quality)
- Bam (PacBio)
- POD5/FAST5 (ONT)

GDC Genetic Diversity Centre Zurich

Sequence Data Format: **Fasta** (**>**)

Start

Unique Sequence Header

```
1 - >BY999847.1 BY999847 Moon Jellyfish cDNA library Aurelia aurita cDNA
    clone Aa_plW_142145_H14, mRNA sequence
2 - AAAATACCGCATGATTGTTCGTTTCACAAACAAAGATATAGCTTGCCAGATAGCGTATGCCAGATTGCAA
3 - GGAGATGTGATCATTTGTGCAGCTTATGCTCATGAACTCCCAAGATATGGTGTCAAGGTCGGGTTGACCA
4 - ACTATGCAGCTGCTTATTGCACTGGCCTCTTGCTCGCAAGAAGGCTCCTTTCAAAATTGAAATTGGCTGA
5 - CACTTACAAAGGTTGTGAAGAAGTGAATGGTGATGAATACCTTGTGGAAGGAGAGGAGGGACAGCCTGGA
6 - CCTTTCCGTTGTTACCTTGATATTGGCCTTGCCAGAACCTCAACTGGTGCCAAGATCTTTGGTGCATTGA
7 - AAGGTGCAGTTGATGGTGGACTTGACATCCCACACAGCAACACGAGATTCCCTGGTTATGACAATGAAGC
8 - AAAGGAATTTGACCCAGAGGTGCACAGACAACACA…
. . .
```

$N_{rows} \geqq 2$ per sequences read

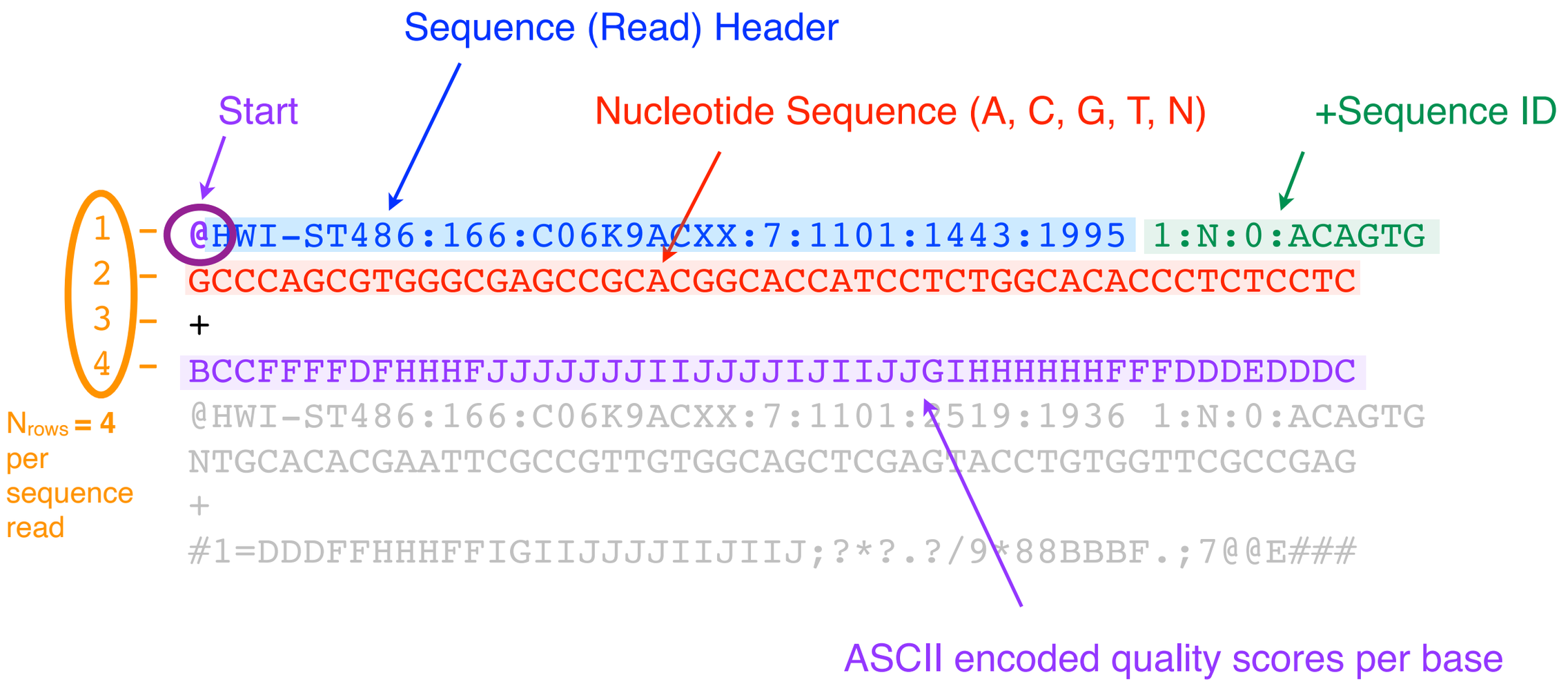
Sequence (nucleotide or protein)

File extension: sequence(s).**fa**, sequence(s).**fasta**

Special cases: sequences.**mfa** (multiple - aligned - sequences)
sequences.**afa** (aligned sequences)

GDC Genetic Diversity Centre Zurich

(Illumina) Sequence Data Format: **Fastq** (@)



File extension: reads.**fq**, reads.**fastq**

Special cases: read_**R**[12].fq (> paired reads)

read_**I**[12].fq (> index reads)

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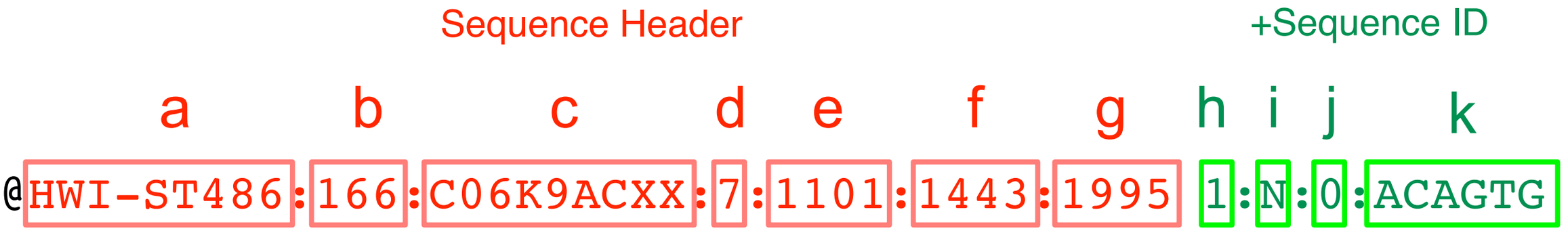
Illumina Fastq Header Format (version > 1.8)



a. **unique instrument name**
b. run id
c. flowcell id
d. flowcell lane
e. tile number within the flowcell lane
f. x-coordinate of the cluster within the tile
g. y-coordinate of the cluster within the tile

h. **the member of a pair, 1 or 2 (paired-end or mate-pair reads only)**
i. Y if the read fails filter (read is bad), N otherwise (read passed filter)
j. 0 when no control bits are on
k. index sequence

Older Illumina Fastq Header Format (version < 1.8)

```
        a          b  c    d     e     f g
@HWUSI-EAS100R:6:73:941:1973#0/1
```

a. unique instrument name
b. flowcell lane
c. tile number within the flowcell lane
d. x-coordinate of the cluster within the tile
e. y-coordinate of the cluster within the tile
f. index number for a multiplexed sample (0 for no indexing)
g. the member of a pair, /1 or /2 *(paired-end or mate-pair reads only)*

GDC
Zurich Centre Diversity Genetic

PacBio Fastq Header Format

BAM → pbtk::bam2fastx → FASTQ

```
@m84151_240325_161603_s2/97453283/ccs bc=24,25 bl=GGTAGCACACGCACTGAGATACAGGAAACAGCTATGAC bq=100
bt=GTCATAGCTGTTTCCTGTACTCTCAGAGACACACTACCAT bx=38,40 cx=12 qe=3280
ql=IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII qs=38 qt=IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
TATCGCTCTTAAACGAGGAATCTCTAGTAAGTGCATTTCATGAAAATGCGCTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATT
GAATGATGTGATGAAAATCTCAGAGATTGTATACGCCAATAGACTGGGCAACTGGTCTATGTATACTGGTCAAAGAGATTTGAATCTTATTATTTAGAGG
AAGGAGAAGTCGTAACAAGGTATCTGTAGGTGAACCTGCAGATGGATCATTCGTTACTAAACCATTTTTTTTATTTTAAACAACCCAATTGTTAATAGTC
TTTTTATAAACCAAACCATTCAATTATTTTTTATTTCAA…
+
IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII…
```

PacBio (Revio) Fastq Header Format

```
@m84151_240325_161603_s2/97453283/ccs - QNAME (query template name)
bc=24,25 - Barcode Calls
bl=GGTAGCACACGCACTGAGATACAGGAAACAGCTATGAC - Barcode sequence clipped from leading end
bq=100 - Barcode Quality [0-100]
bt=GTCATAGCTGTTTCCTGTACTCTCAGAGACACACTACCAT - Barcode sequence clipped from trailing end
bx=38,40 - Pair of clipped barcode sequence lengths
cx=12 - subread tag
qe=3280 - For CCS reads, the 0-based end of the query in its original CCS read.
ql=IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII - Qualities of bc bases clipped from leading end
qs=38 - For CCS reads, the 0-based start of the query in its original CCS read.
qt=IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII - Qualities of bc bases clipped from trailing end
```

The `QNAME` for by-strand CCS reads includes a suffix **`fwd`** or **`rev`** to indicate strand relative to the other by-strand read for the ZMW. Strand assignment by CCS is arbitrary and does not imply the strand that may be assigned during mapping.

Source: https://pacbiofileformats.readthedocs.io/en/13.0/index.html

ONT Fastq Header Format

```
@c66cf9d5-8ceb-49a2-87ba-573bdf744476 runid=da30afcc85dd427c946e129369e698f701722998 read=19 ch=21
start_time=2023-12-13T17:44:43.197441+01:00 flow_cell_id=FAY08440
protocol_group_id=20231213_LakeGeneva_Sasi sample_id=LG1_Stepeffluent
parent_read_id=c66cf9d5-8ceb-49a2-87ba-573bdf744476
basecall_model_version_id=dna_r10.4.1_e8.2_400bps_sup@v4.2.0
ATCATATTTACTTCGTTCAGTTACGTATTGCTGTTTCAATCTTGGTGAAATTAATTCTGTTTGATTATTGTTTCCATCTTGTTTACTTTCACCATAATTA
AAATATAAAATTTCTTTATATAATGTTTCTTTTGAATAGTTTTCTATTAAAAAGCTTTTGTCATCAATATTATTATTTTTATATTCTTCAAATAATTTTA
AAAATAAATTGTAATCAGTTTTATTAAATTGATAGCAATACATTA
+
#$$#$$%'('*/001:<;?
<<;920000==:::;;QHIHHKLFKSSNSKNJMF?;78888??;BILGKJIKRMHHHSGSJKKSHLPSISLSSMKNLHJIPHSHHPSPMSHNSSMQSSPM
NKJHGJSSSNSHIPLJHIKSOHKLJHHGJSGOLILSMMSNIL9999;ISNKMS99999SKSHSSISJSIHIMKSHJLSSNMSKHLSSMKKKIIHKSPMJG
KSSJSSJSJSIKSIGIJNJEFJ755)
```

The headers in FASTQ files generated by Oxford Nanopore Technologies (ONT) basecallers are defined by the specific basecaller used (such as Dorado, Guppy, Albacore, Bonito, etc.) and have changed many times over the years.

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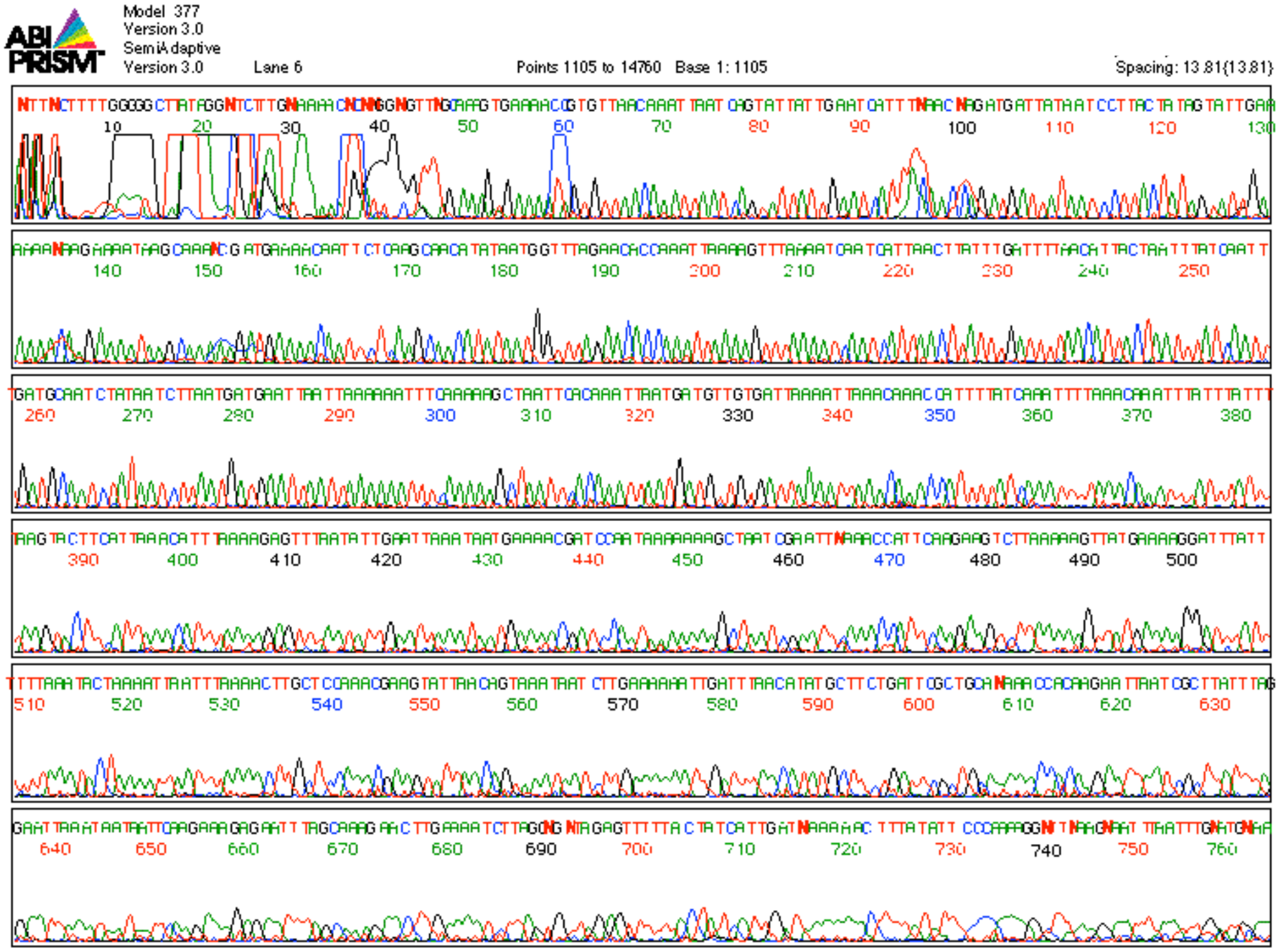
ONT Fastq Header Format

```
@c66cf9d5-8ceb-49a2-87ba-573bdf744476
runid=da30afcc85dd427c946e129369e698f701722998
read=19
ch=21
start_time=2023-12-13T17:44:43.197441+01:00
flow_cell_id=FAY08440
protocol_group_id=p1023_run240512_Meta
sample_id=Shower-123sw
parent_read_id=c66cf9d5-8ceb-49a2-87ba-573bdf744476
basecall_model_version_id=dna_r10.4.1_e8.2_400bps_sup@v4.2.0
```

The FASTQ headers generated by Dorado, like those from other ONT basecallers, are designed to provide comprehensive metadata about each read. Understanding the specific format used by the version of Dorado you are working with is critical to effective data management and analysis.

Sequence QC


Model 377
Version 3.0
SemiAdaptive
Version 3.0
Lane 6
Points 1105 to 14760 Base 1: 1105
Spacing: 13.81(13.81)

Fastq Sequence Read

```
1 - @HWI-ST486:166:C06K9ACXX:7:1101:1443:1995 1:N:0:ACAGTG
2 - ACTGAGCGTGGGCGAGCCGCACGGCACCATCCTCTGGCACACCCTCTCCTC
3 - +
4 - 5576FFFDFHHHFJJJJJJJIIJJJJIJIIJJGIHHHHHHFFFDDDEDDDC
```

⇧

ASCII **encoded** quality scores per base

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Sequencing (phred) **quality scores (Q)** measures the (error) **probability (P)** that a base is called (in)correctly.

position	1	2	3	4	...
nucleotide	A	C	G	T	...
quality score (Q)	20	20	22	21	...

https://www.phrap.com/phred/

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Sequencing (phred) **quality scores (Q)** measure the (error) **probability (P)** that a base is called incorrectly.

Base-Calling Error Probability

$$P = 10^{\frac{-Q}{10}}$$

Phred Quality Score

$$Q = -10 \log_{10} P$$

Sequencing (phred) **quality scores (Q)** measures the (error) **probability (P)** that a base is called incorrectly.

position	1	2	3	4	...
nucleotide	A	C	G	T	...
quality score (Q)	20	20	22	21	...

$$P = 10^{\frac{-Q}{10}} = 10^{-2} = 0.01 \rightarrow 1\% \ (\rightarrow 99\%)$$

Sequencing (phred) **quality scores (Q)** measure the (error) **probability (P)** that a base is called incorrectly.

position	1	2	3	4	...
nucleotide	A	C	G	T	...
quality score (Q)	20	20	22	21	...
probability (P)	0.01	0.01	0.006	0.008	...
accuracy (1-P)	0.99	0.99	0.994	0.992	...

One character encoding!

1	2	3	4
A	C	G	T
20	20	22	21

1	2	3	4		1	2	3	4		1	2	3	4
A	C	G	T	or	A	C	G	T	or	A	C	G	T
a	a	c	b		+	+	*	"		😏	😏	😜	😀

20→a 21→b 22→c

20→+ 21→" 22→*

20→😏 21→😀 22→😜

ASCII TABLE

Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char	Decimal	Hex	Char
0	0	[NULL]	32	20	[SPACE]	64	40	@	96	60	`
1	1	[START OF HEADING]	33	21	!	65	41	A	97	61	a
2	2	[START OF TEXT]	34	22	"	66	42	B	98	62	b
3	3	[END OF TEXT]	35	23	#	67	43	C	99	63	c
4	4	[END OF TRANSMISSION]	36	24	$	68	44	D	100	64	d
5	5	[ENQUIRY]	37	25	%	69	45	E	101	65	e
6	6	[ACKNOWLEDGE]	38	26	&	70	46	F	102	66	f
7	7	[BELL]	39	27	'	71	47	G	103	67	g
8	8	[BACKSPACE]	40	28	(	72	48	H	104	68	h
9	9	[HORIZONTAL TAB]	41	29	)	73	49	I	105	69	i
10	A	[LINE FEED]	42	2A	*	74	4A	J	106	6A	j
11	B	[VERTICAL TAB]	43	2B	+	75	4B	K	107	6B	k
12	C	[FORM FEED]	44	2C	,	76	4C	L	108	6C	l
13	D	[CARRIAGE RETURN]	45	2D	-	77	4D	M	109	6D	m
14	E	[SHIFT OUT]	46	2E	.	78	4E	N	110	6E	n
15	F	[SHIFT IN]	47	2F	/	79	4F	O	111	6F	o
16	10	[DATA LINK ESCAPE]	48	30	0	80	50	P	112	70	p
17	11	[DEVICE CONTROL 1]	49	31	1	81	51	Q	113	71	q
18	12	[DEVICE CONTROL 2]	50	32	2	82	52	R	114	72	r
19	13	[DEVICE CONTROL 3]	51	33	3	83	53	S	115	73	s
20	14	[DEVICE CONTROL 4]	52	34	4	84	54	T	116	74	t
21	15	[NEGATIVE ACKNOWLEDGE]	53	35	5	85	55	U	117	75	u
22	16	[SYNCHRONOUS IDLE]	54	36	6	86	56	V	118	76	v
23	17	[ENG OF TRANS. BLOCK]	55	37	7	87	57	W	119	77	w
24	18	[CANCEL]	56	38	8	88	58	X	120	78	x
25	19	[END OF MEDIUM]	57	39	9	89	59	Y	121	79	y
26	1A	[SUBSTITUTE]	58	3A	:	90	5A	Z	122	7A	z
27	1B	[ESCAPE]	59	3B	;	91	5B	[	123	7B	{
28	1C	[FILE SEPARATOR]	60	3C	<	92	5C	\	124	7C	|
29	1D	[GROUP SEPARATOR]	61	3D	=	93	5D	]	125	7D	}
30	1E	[RECORD SEPARATOR]	62	3E	>	94	5E	^	126	7E	~
31	1F	[UNIT SEPARATOR]	63	3F	?	95	5F	_	127	7F	[DEL]

Illumina Quality Encoding (version > 1.8+)

Decimal	Hex	Char	Decimal	Hex	Char
32	20	[SPACE]	64	40	@
33	21	!	65	41	A
34	22	"	66	42	B
35	23	#	67	43	C
36	24	$	68	44	D
37	25	%	69	45	E
38	26	&	70	46	F
39	27	'	71	47	G
40	28	(	72	48	H
41	29	)	73	49	I
42	2A	*	74	4A	J
43	2B	+	75	4B	K
44	2C	,	76	4C	L
45	2D	-	77	4D	M
46	2E	.	78	4E	N
47	2F	/	79	4F	O
48	30	0	80	50	P
49	31	1	81	51	Q
50	32	2	82	52	R
51	33	3	83	53	S
52	34	4	84	54	T
53	35	5	85	55	U
54	36	6	86	56	V
55	37	7	87	57	W
56	38	8	88	58	X
57	39	9	89	59	Y
58	3A	:	90	5A	Z
59	3B	;	91	5B	[
60	3C	<	92	5C	\
61	3D	=	93	5D	]
62	3E	>	94	5E	^
63	3F	?	95	5F	_

$$Q + 33 = ASCII$$

$$20 + 33 = 53 \rightarrow 5$$

position	1	2	3	4	...
nucleotide	A	C	G	T	...
quality score Q	20	20	22	21	...
Ascii	53	53	55	54	
char endcoding	5	5	7	6	...

```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................
 ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX..........................
 ..................................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII..........................
 ..................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ..........................
 ..LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
 |                         |    |        |                              |                     |
33                        59   64       73                            104                   126
 0........................26...31.......40
                         -5....0........9.............................40
                              0........9.............................40
                                 3.....9.............................40
 0.2......................26...31.......41

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

Function

```
# ascii character > decimal value
asc <- function(x) {
          strtoi(charToRaw(x),16L)
          }
asc("!")
```

```
# decimal value > ascii character
chr <- function(n) {
          rawToChar(as.raw(n))
          }
chr("33")
```

Encoding	ASCII	Q	P
J	74	41	0.00008
I	73	40	0.00010
H	72	39	0.00013
G	71	38	0.00016
F	70	37	0.00020
E	69	36	0.00025
D	68	35	0.00032
C	67	34	0.00040
B	66	33	0.00050
A	65	32	0.00063
@	64	31	0.00079
?	63	30	0.00100
>	62	29	0.00126
=	61	28	0.00158
<	60	27	0.00200
;	59	26	0.00251
:	58	25	0.00316
9	57	24	0.00398
8	56	23	0.00501
7	55	22	0.00631
6	54	21	0.00794
5	53	20	0.01000
4	52	19	0.01259
3	51	18	0.01585
2	50	17	0.01995
1	49	16	0.02512
0	48	15	0.03162
/	47	14	0.03981
.	46	13	0.05012
-	45	12	0.06310
,	44	11	0.07943
+	43	10	0.10000
*	42	9	0.12589
)	41	8	0.15849
(	40	7	0.19953
	39	6	0.25119
&	38	5	0.31623
%	37	4	0.39811
$	36	3	0.50119
#	35	2	0.63096
"	34	1	0.79433
!	33	0	1.00000

Phred Quality Score

$$Q = -10 \log_{10} P$$

Base-Calling Error Probability

$$P = 10^{\frac{-Q}{10}}$$

Q	P	Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%

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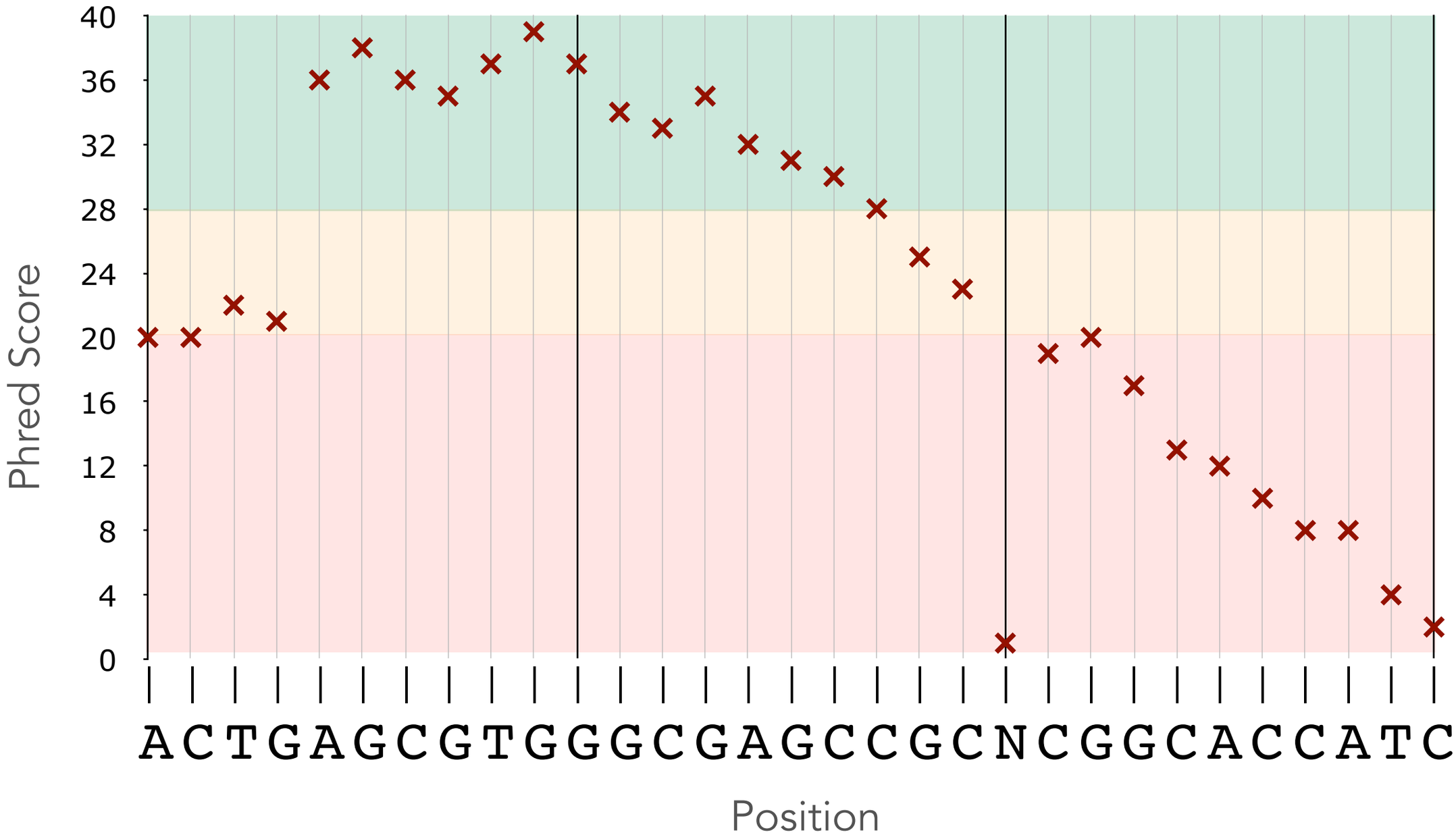
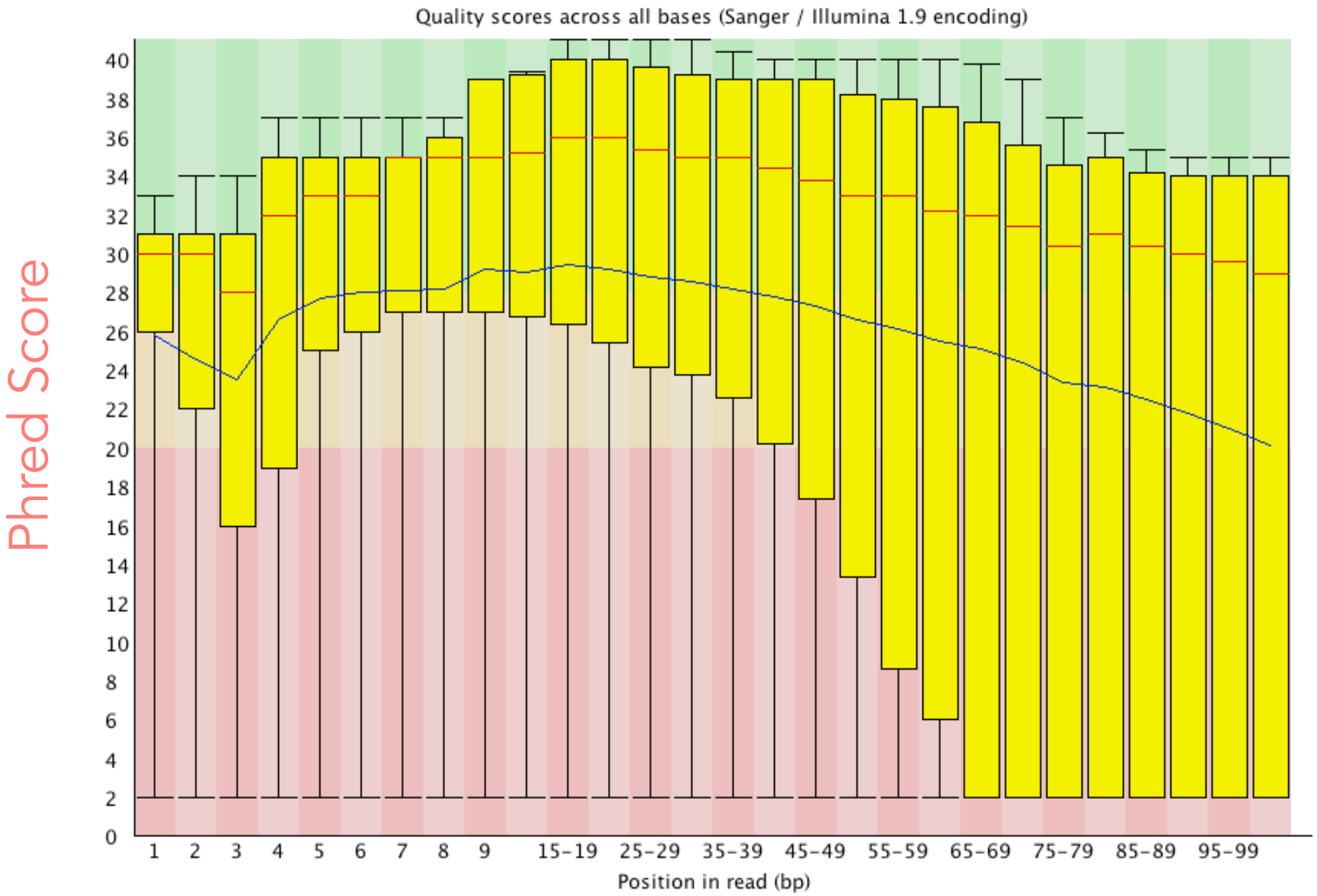
Phred Scores per Base




Quality scores across all bases (Sanger / Illumina 1.9 encoding)
Phred Score
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
15-19
25-29
35-39
45-49
55-59
65-69
75-79
85-89
95-99
Position in read (bp)

MPS ▷ Quality Control

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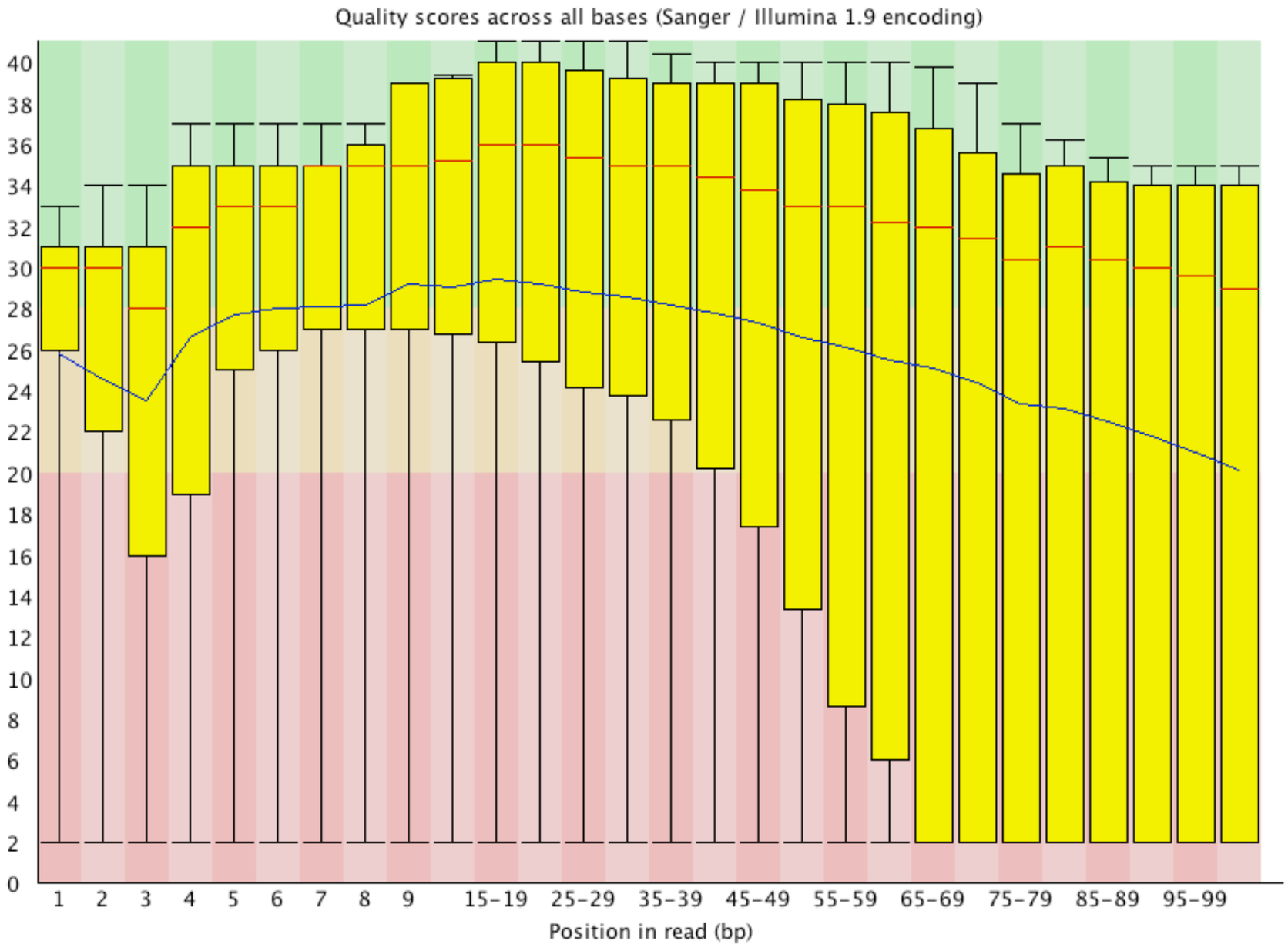
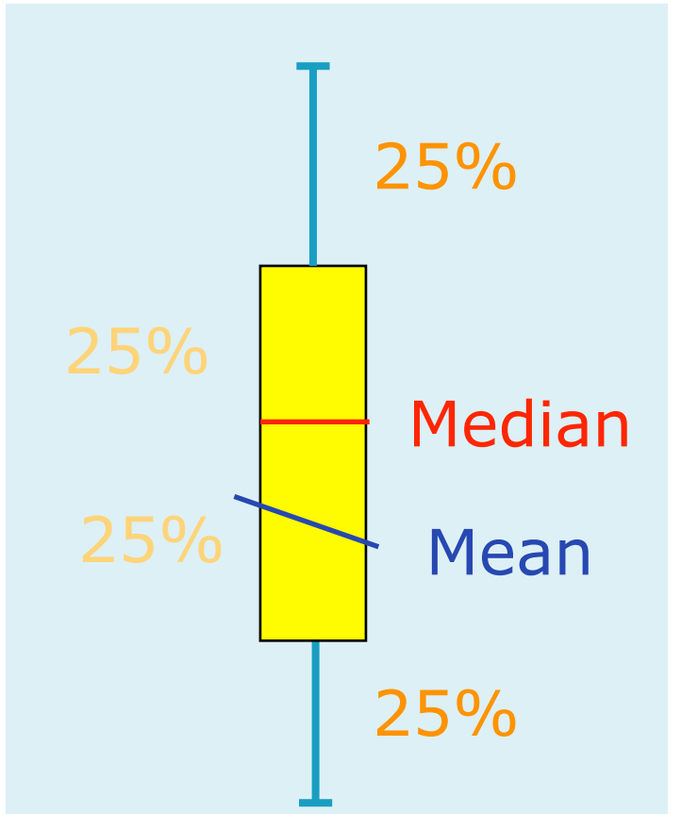


Boxplot

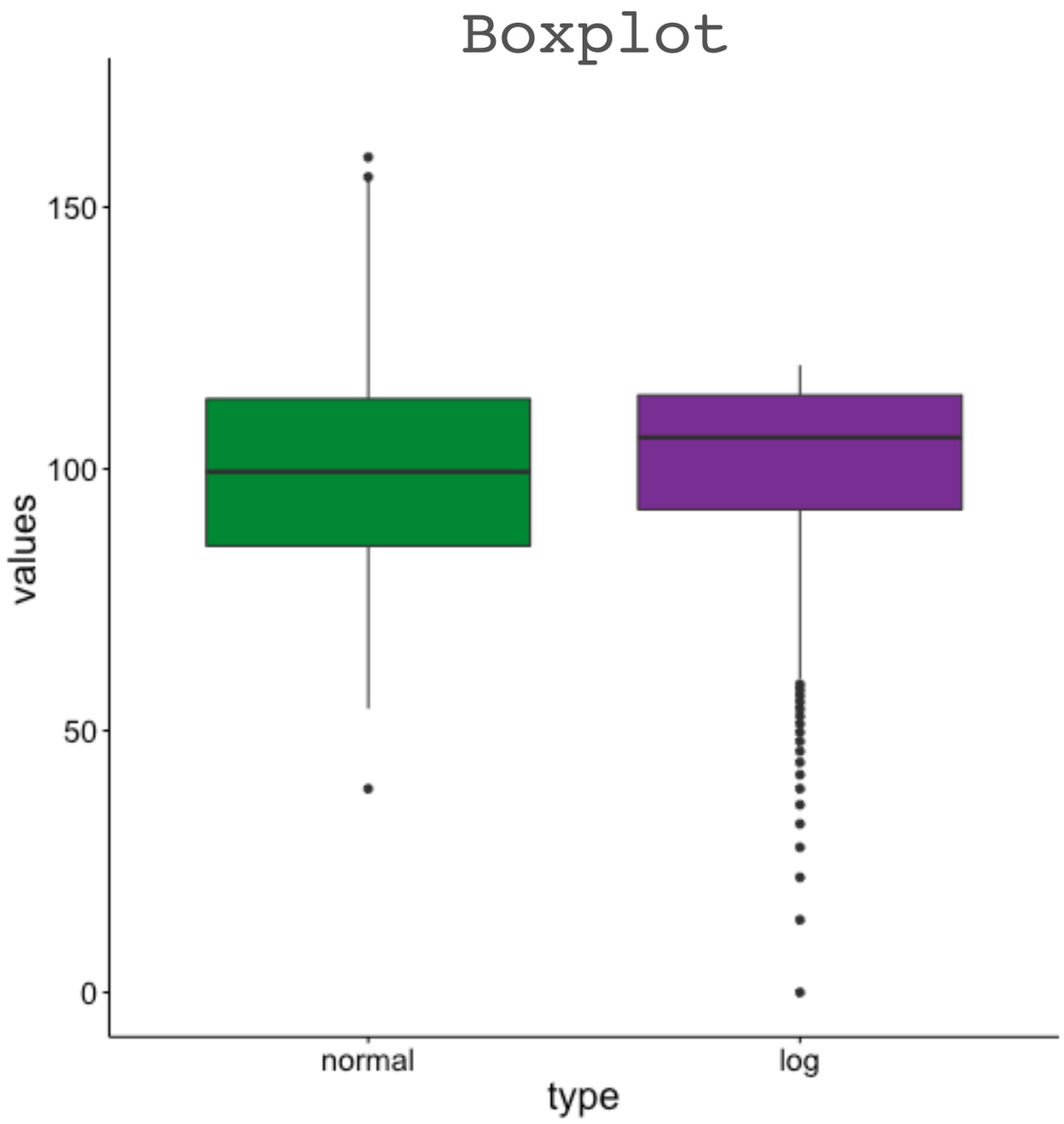


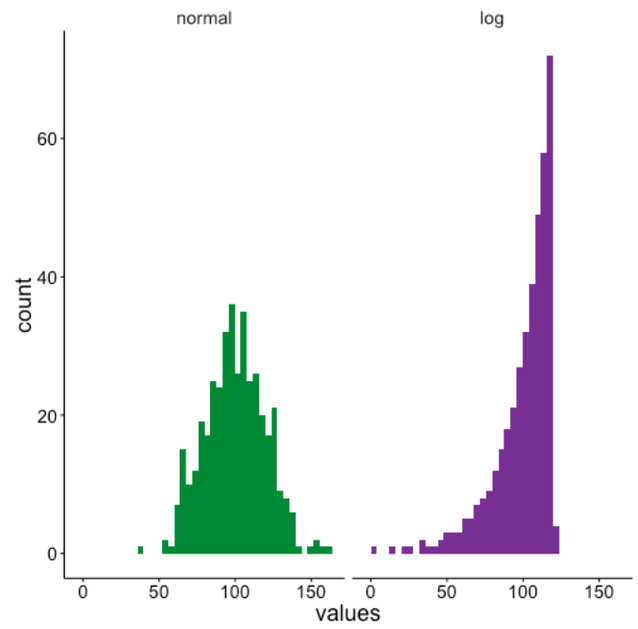
Why do we use a box plot and not a bar plot?

Genetic Diversity Centre Zurich

Why do we use a box plot and not a bar plot?

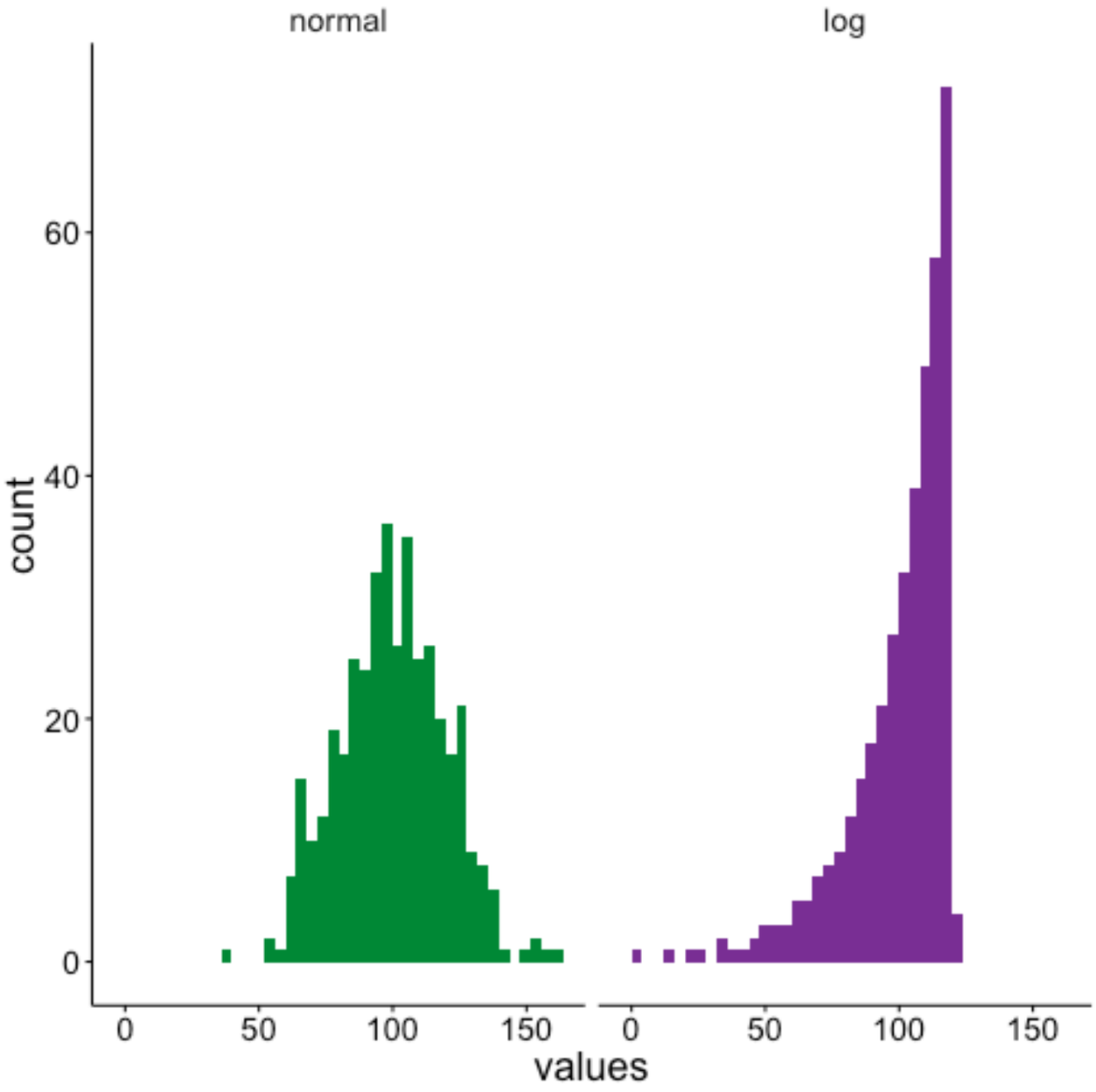
Box plots are generally more informative than bar charts, especially when you need to understand the distribution and variability of your data.





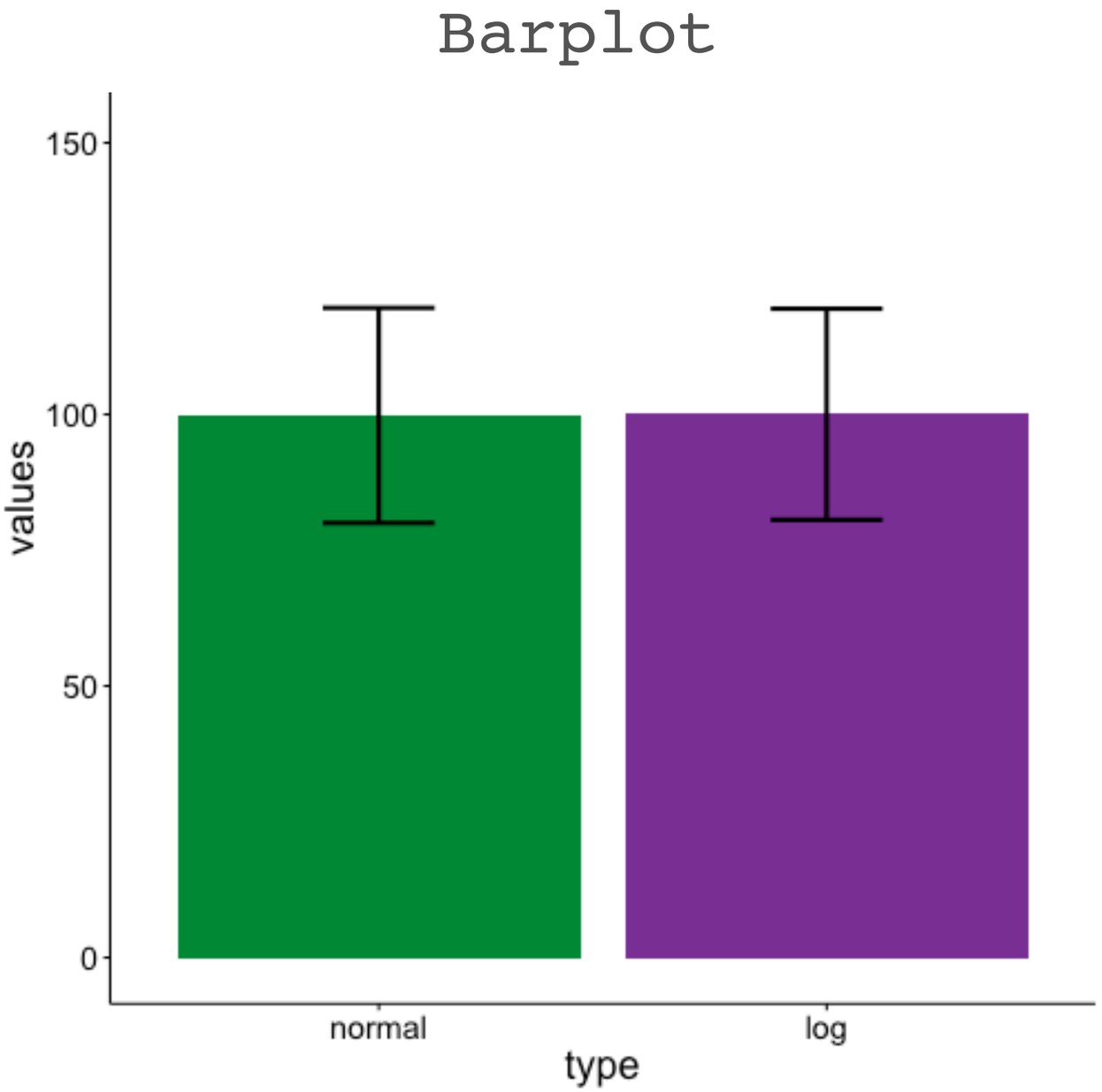
https://pagepiccinini.com/2016/02/23/boxplots-vs-barplots/

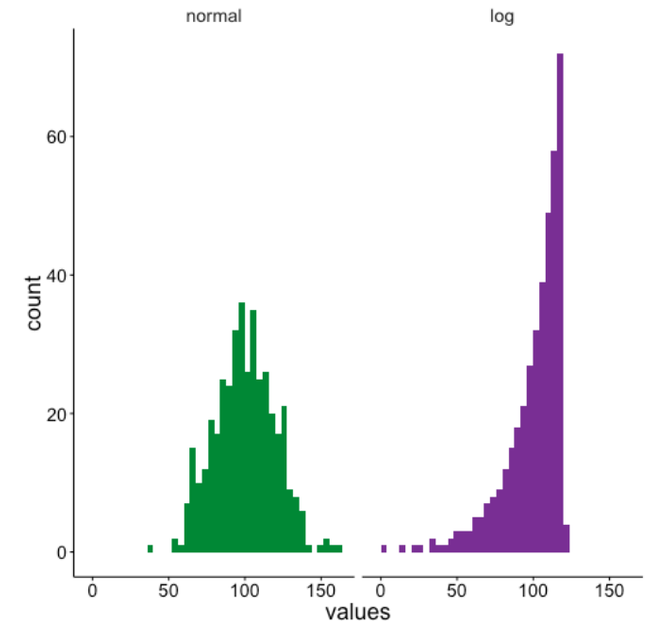
Histogram

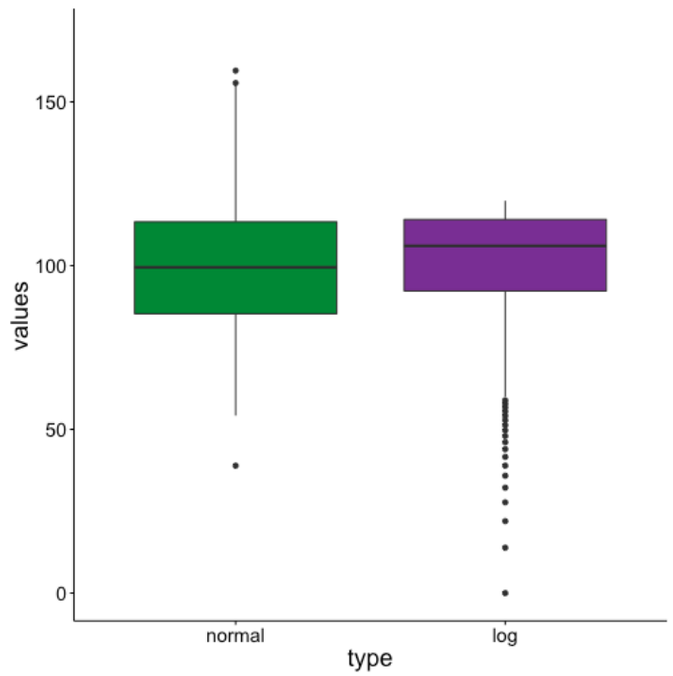


mean:100
sd: 20

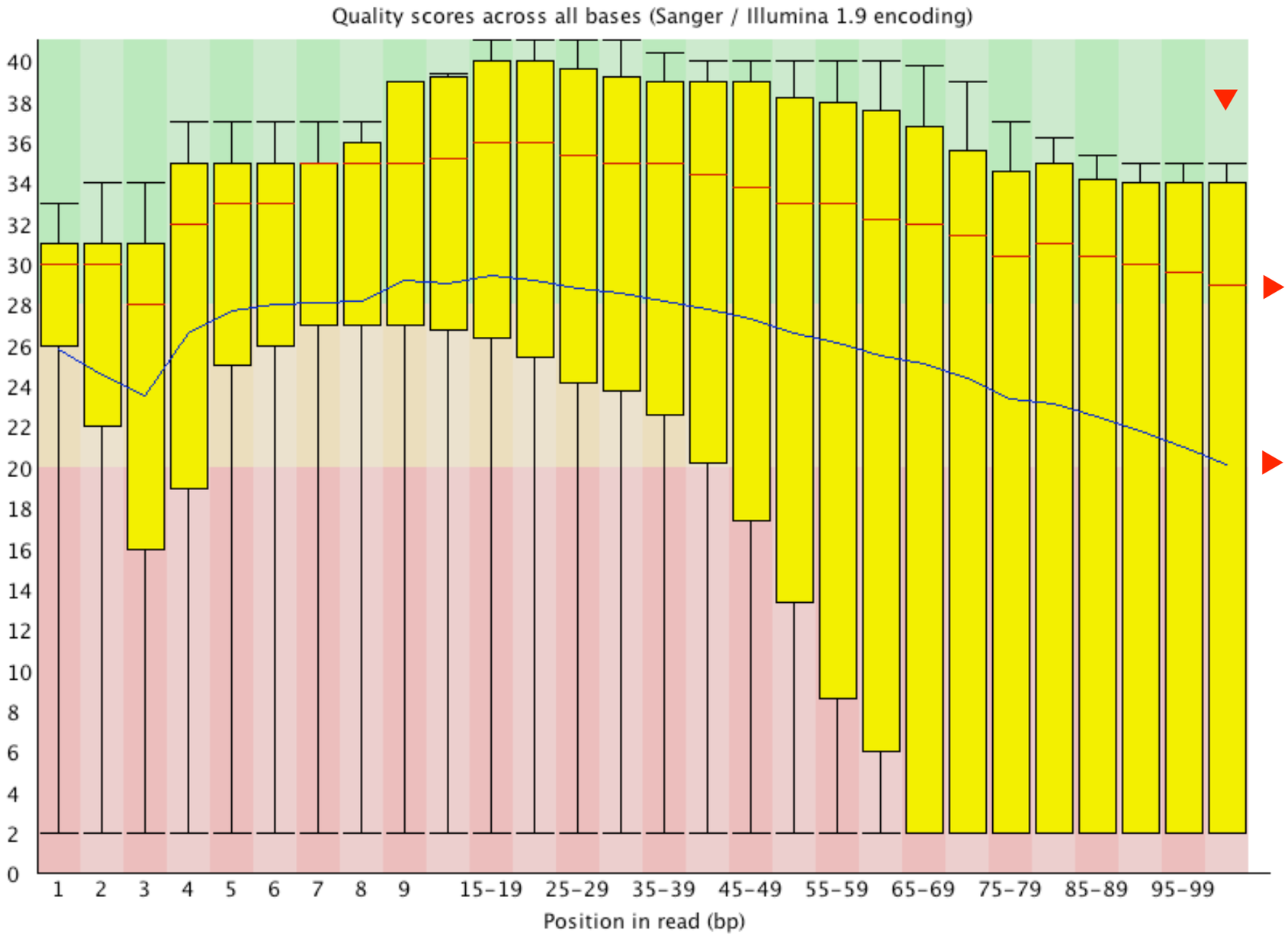
https://pagepiccinini.com/2016/02/23/boxplots-vs-barplots/







https://pagepiccinini.com/2016/02/23/boxplots-vs-barplots/



- $Q_{(median)}$ = 30 → 99.9%
- $Q_{(mean)}$ = 20 → 99%

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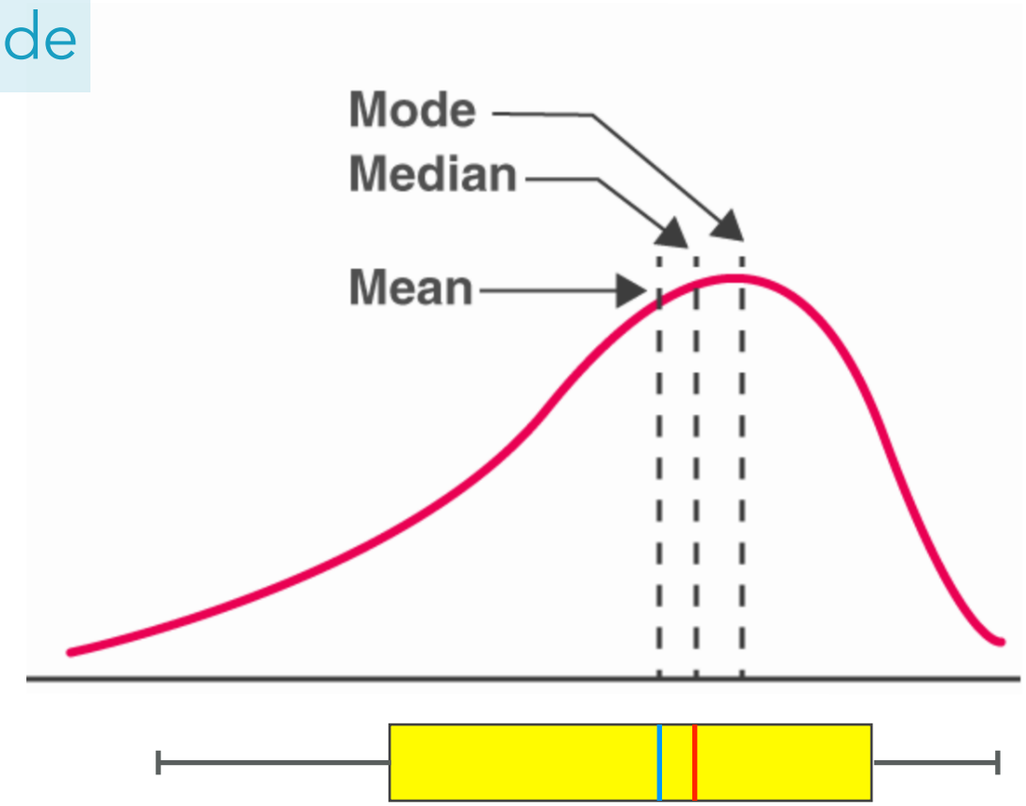
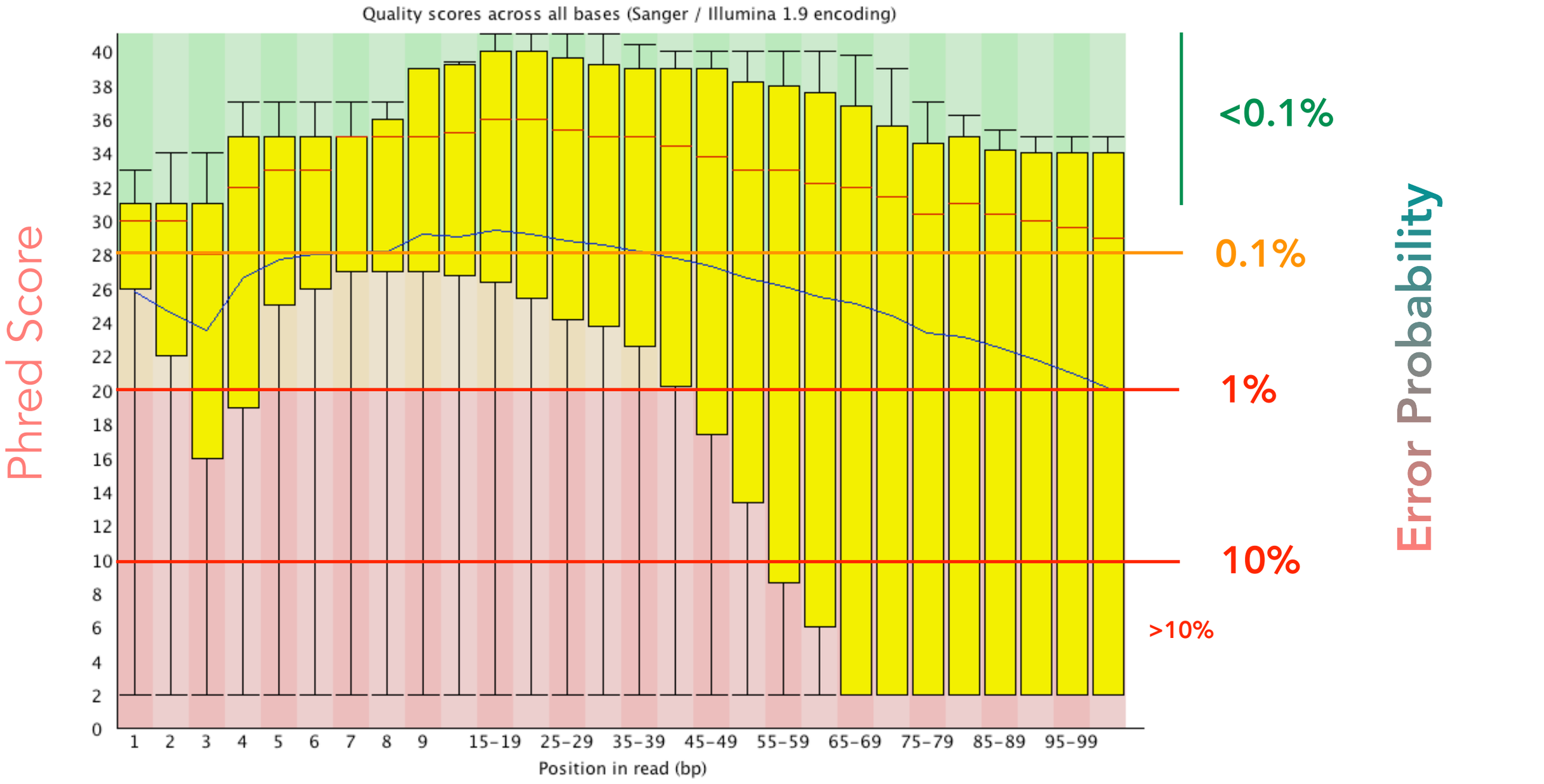
Symmetrical Distribution

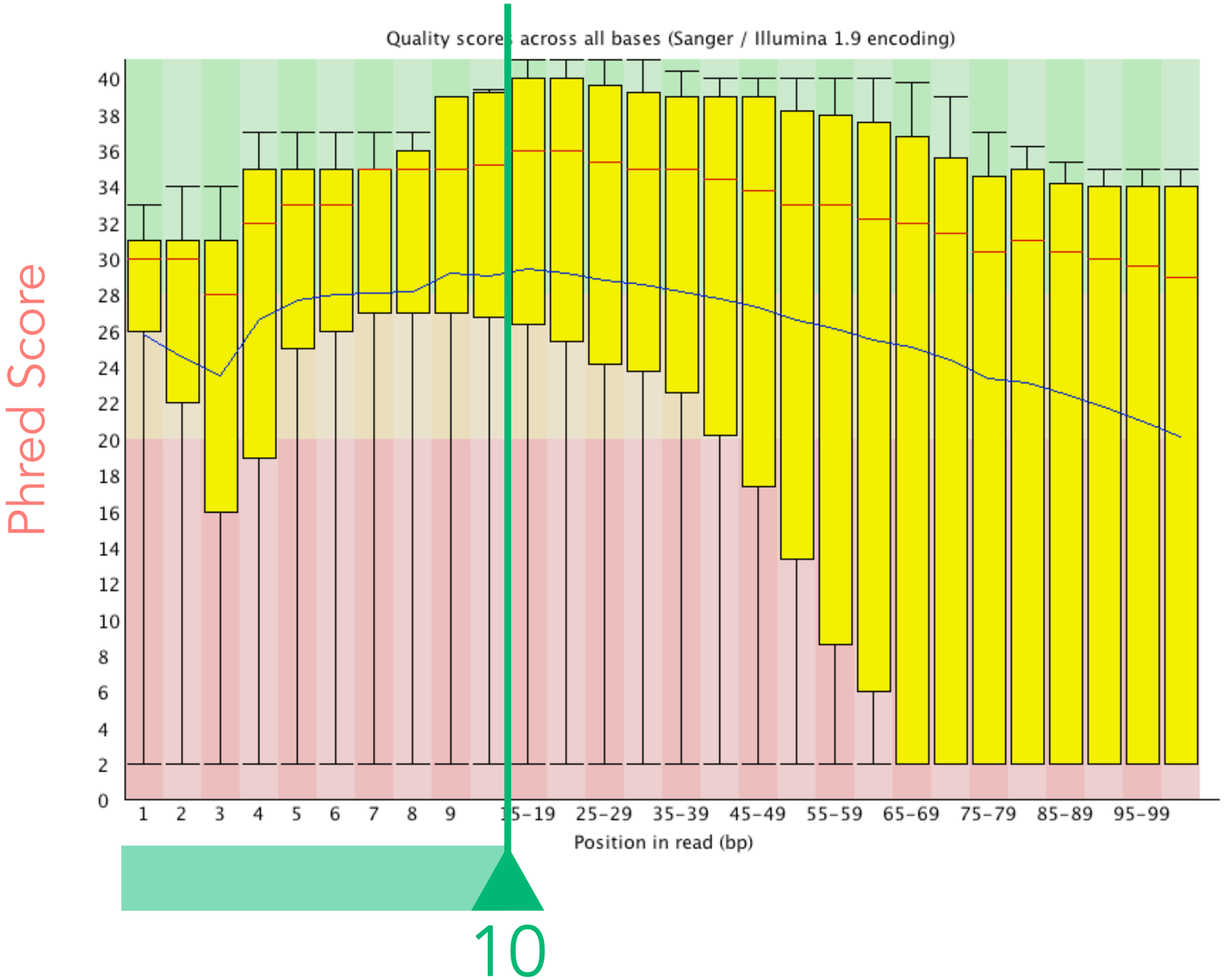
mean = median = mode

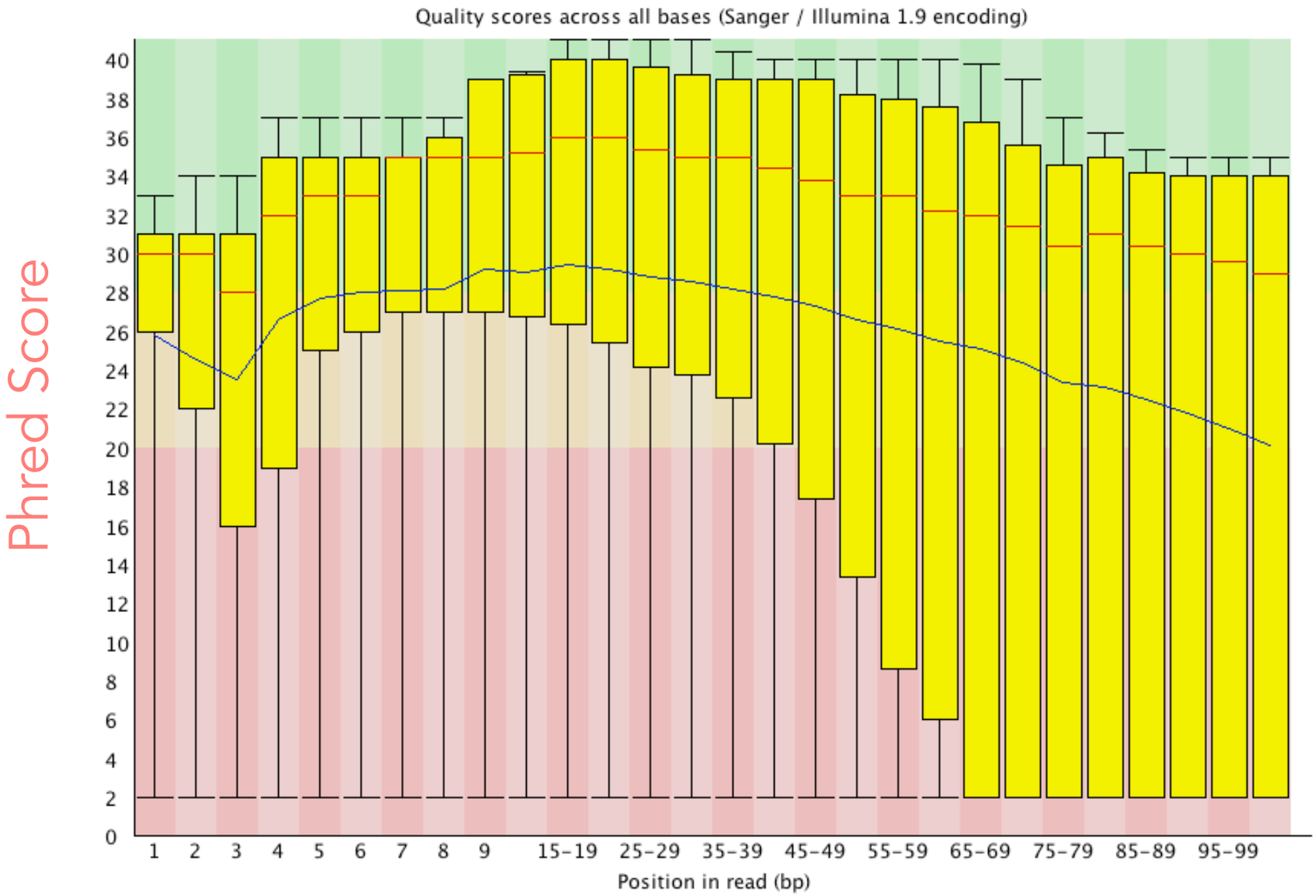


mean < median

skewed to the left


Quality scores across all bases (Sanger / Illumina 1.9 encoding)
Phred Score
Error Probability
<0.1%
0.1%
1%
10%
>10%
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
15-19
25-29
35-39
45-49
55-59
65-69
75-79
85-89
95-99
Position in read (bp)


Quality scores across all bases (Sanger / Illumina 1.9 encoding)
Phred Score
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
15-19
25-29
35-39
45-49
55-59
65-69
75-79
85-89
95-99
Position in read (bp)
10



Note: Error rate increases along the length of the read.

$Q_{mean\ phred\ score}$: 30

150nt

% Q-score >= Q30 (percentage of bases that have a Q-score above or equal to 30; Q30 is a probability of incorrect base calling of 1 in 1000).

$Q_{mean} = 30$

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Q_{mean} =30

Q_{mean} =30

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Q_{mean} =30

Q_{mean} =30

Q_{mean} =30

Q20 - 150nt

$$0.99^{150} \approx e^{-150\times0.01} = e^{-1.5} \approx 0.2215$$

Approximately 22% of the reads are expected to have no errors at all.

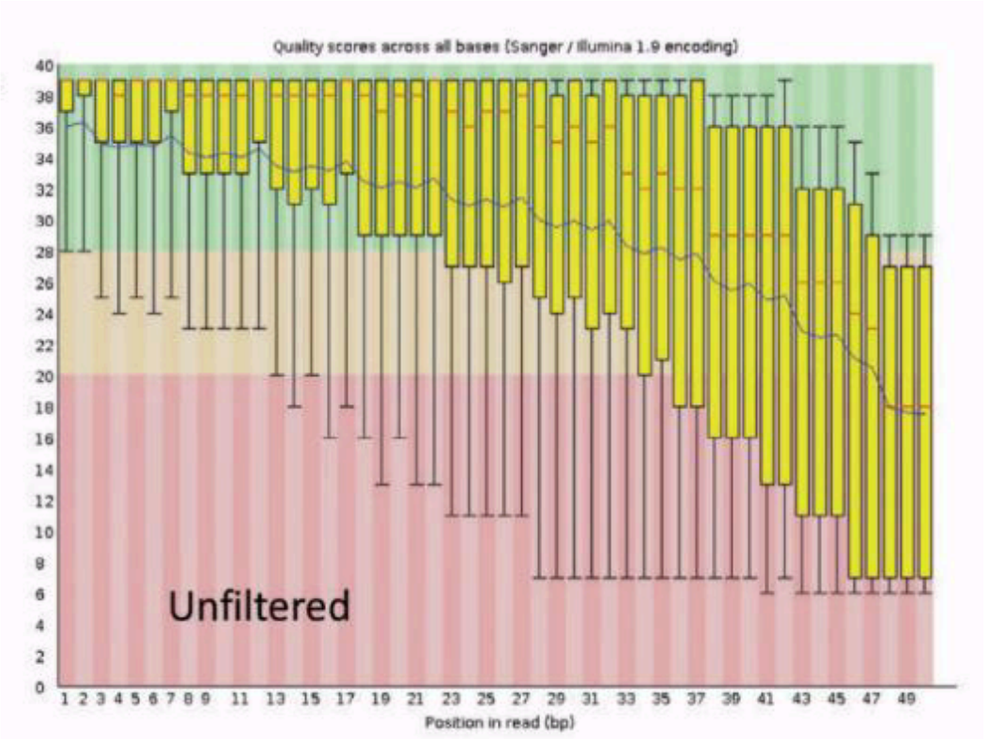
Q20 - 2 x 150nt

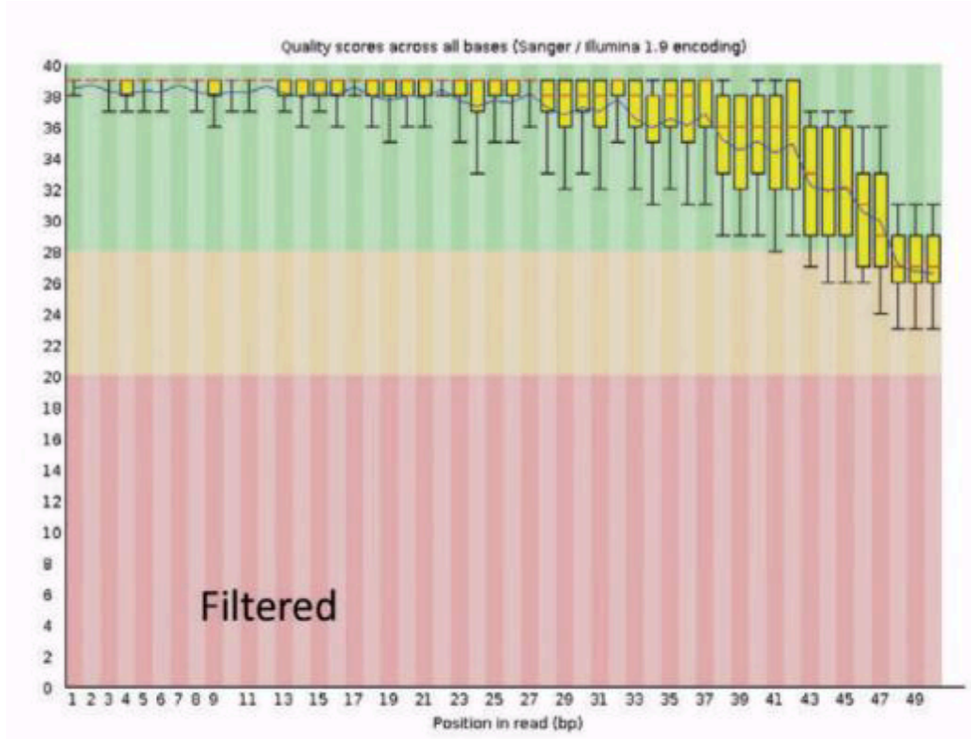
$$0.99^{2*150} \approx e^{-300\times0.01} = e^{-3} \approx 0.0490$$

Approximately 5% of the paired-end reads are expected to have no errors at all.

Q	Read_Length	%_Error_Free
Q20	150	22%
Q30	150	86%
Q40	150	99%
Q40	300	97%
Q40	1500	86%
Q40	5000	61%

For Better or Worse



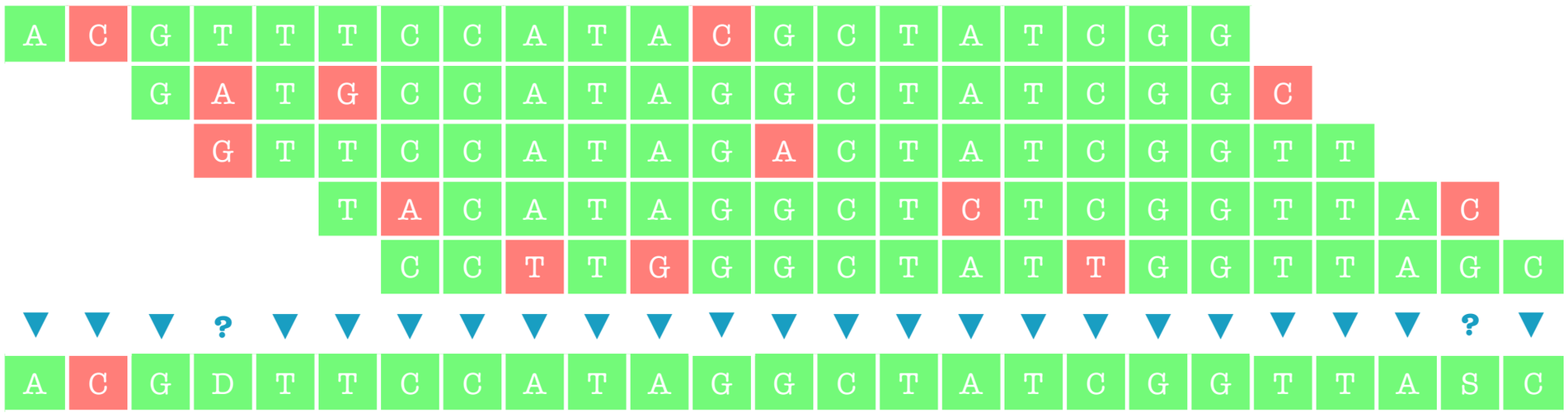


N_{reads} = 6Mio

N_{reads} = 2.5Mio (-60%)

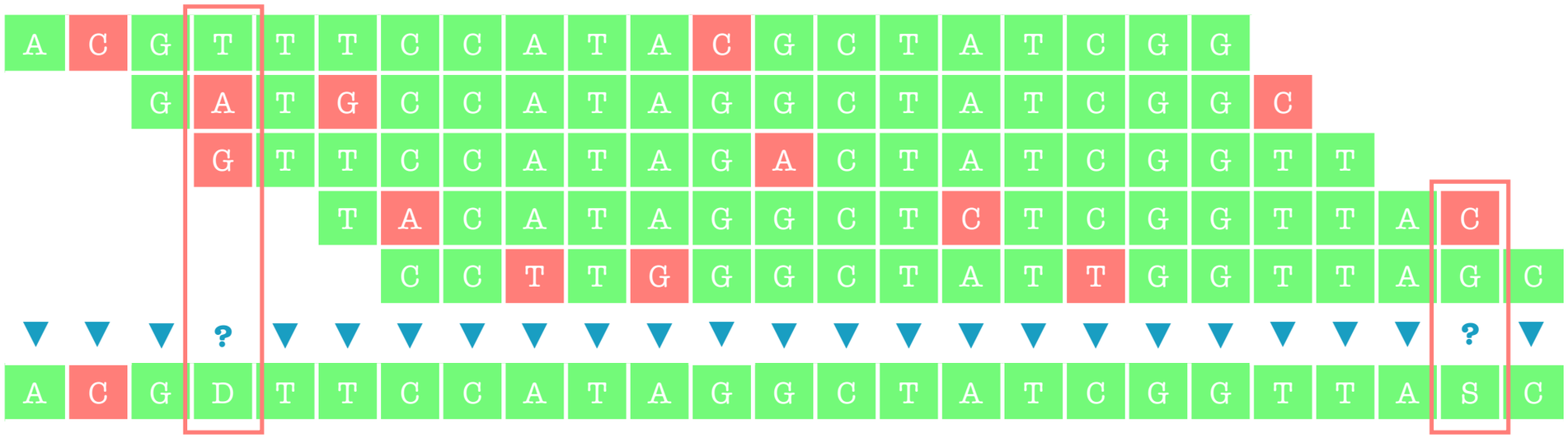
Sequencing Error Rate

Error Correction



- Read quality
- Number of reads (coverage)

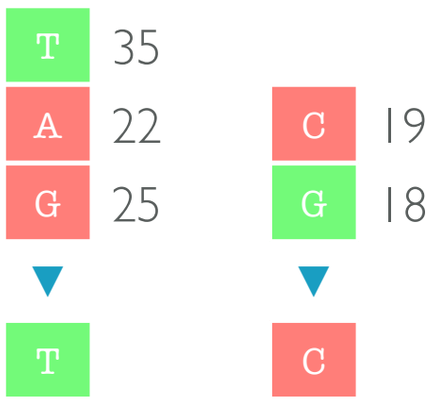
Error Correction



Read quality

Number of reads (coverage)

Phred score



Schirmer *et al. BMC Bioinformatics* (2016) 17:125
DOI 10.1186/s12859-016-0976-y

BMC Bioinformatics

RESEARCH ARTICLE Open Access

Illumina error profiles: resolving fine-scale variation in metagenomic sequencing data

Melanie Schirmer[1,2,4*], Rosalinda D'Amore[3], Umer Z. Ijaz[4], Neil Hall[3] and Christopher Quince[5]

Abstract

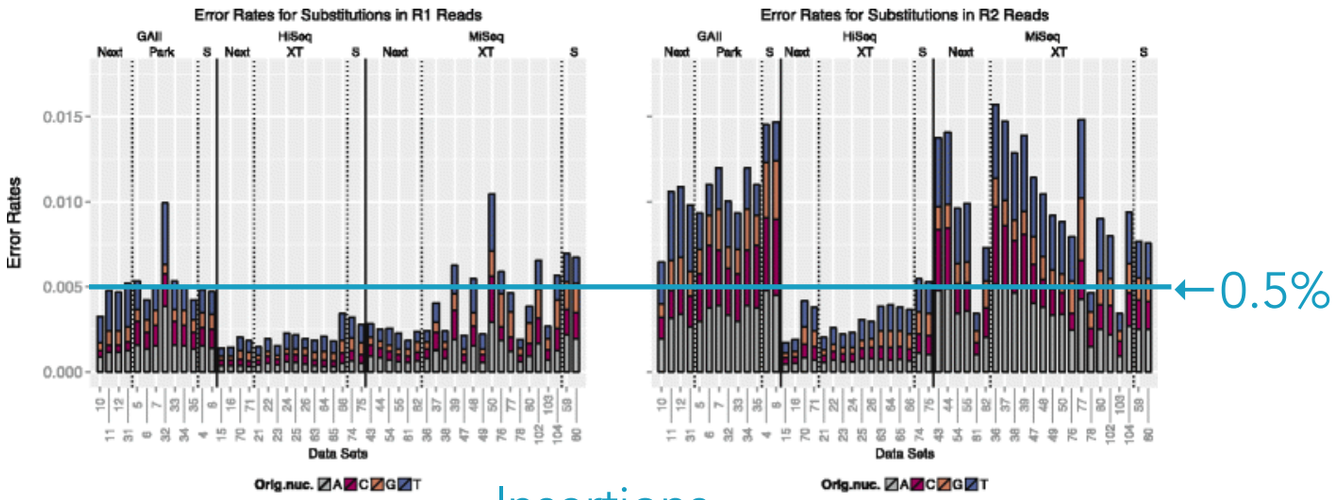
Background: Illumina's sequencing platforms are currently the most utilised sequencing systems worldwide. The technology has rapidly evolved over recent years and provides high throughput at low costs with increasing read-lengths and true paired-end reads. However, data from any sequencing technology contains noise and our understanding of the peculiarities and sequencing errors encountered in Illumina data has lagged behind this rapid development.

Results: We conducted a systematic investigation of errors and biases in Illumina data based on the largest collection of in vitro metagenomic data sets to date. We evaluated the Genome Analyzer II, HiSeq and MiSeq and tested state-of-the-art low input library preparation methods. Analysing in vitro metagenomic sequencing data allowed us to determine biases directly associated with the actual sequencing process. The position- and nucleotide-specific analysis revealed a substantial bias related to motifs (3mers preceding errors) ending in "GG". On average the top three motifs were linked to 16 % of all substitution errors. Furthermore, a preferential incorporation of ddGTPs was recorded. We hypothesise that all of these biases are related to the engineered polymerase and ddNTPs which are intrinsic to any sequencing-by-synthesis method. We show that quality-score-based error removal strategies can on average remove 69 % of the substitution errors - however, the motif-bias remains.

Conclusion: Single-nucleotide polymorphism changes in bacterial genomes can cause significant changes in phenotype, including antibiotic resistance and virulence, detecting them within metagenomes is therefore vital. Current error removal techniques are not designed to target the peculiarities encountered in Illumina sequencing data and other sequencing-by-synthesis methods, causing biases to persist and potentially affect any conclusions drawn from the data. In order to develop effective diagnostic and therapeutic approaches we need to be able to **identify systematic sequencing errors and distinguish these errors from true genetic variation**.

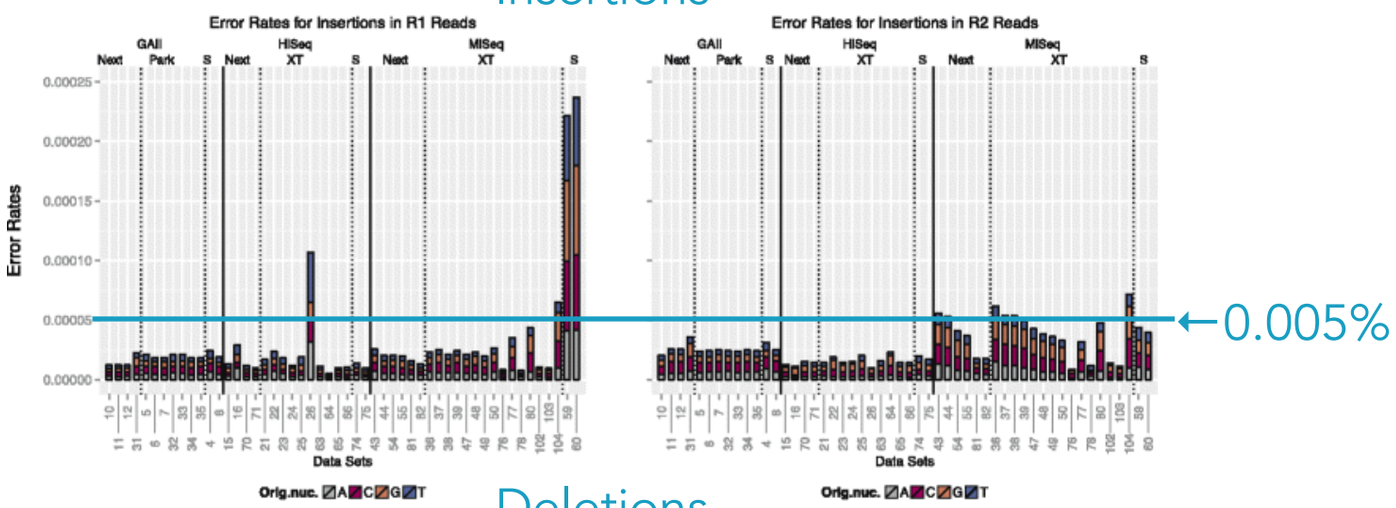
MPS ▷ Quality Control

Substitutions



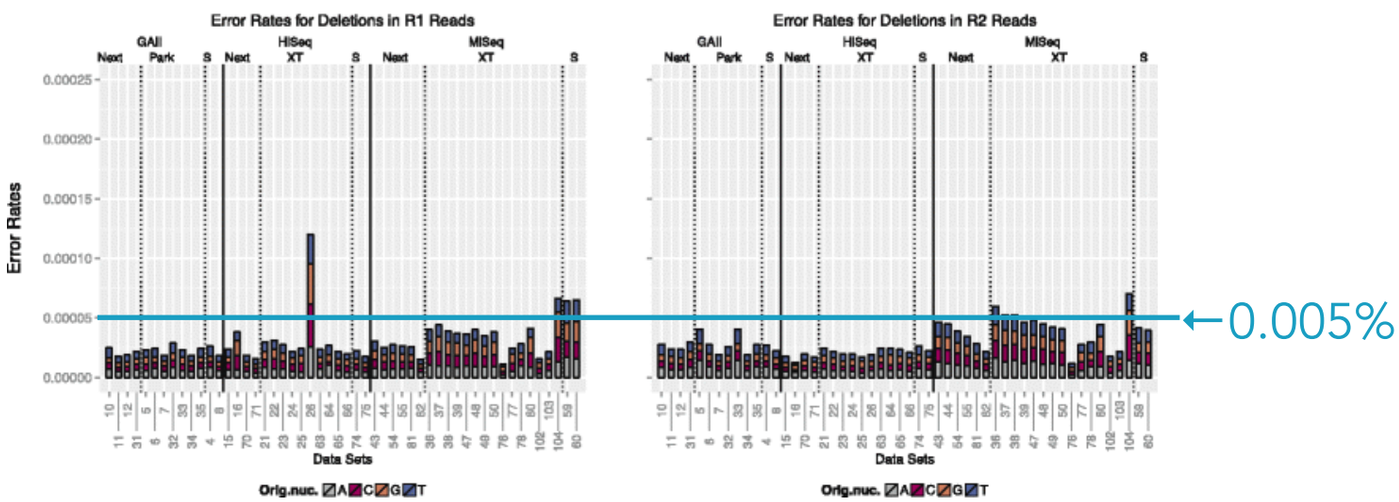
←0.5%

Insertions



←0.005%

Deletions



←0.005%

Illumina

Average substitution rates

Platform	R1/R2	A	C	G	T
GAII	R1	0.0015	0.0010	0.0008	0.0018
GAII	R2	0.0035	0.0029	0.0019	0.0026
HiSeq	R1	0.0004	0.0004	0.0004	0.0008
HiSeq	R2	0.0007	0.0007	0.0007	0.0012
MiSeq	R1	0.0012	0.0009	0.0009	0.0012
MiSeq	R2	0.0033	0.0021	0.0015	0.0031

EE(R1) < EE(R2)

EE(Sub) > EE(Ins) ≈ EE(Del)

EE(HiSeq) < EE(MiSeq) ≈ EE(GAII)

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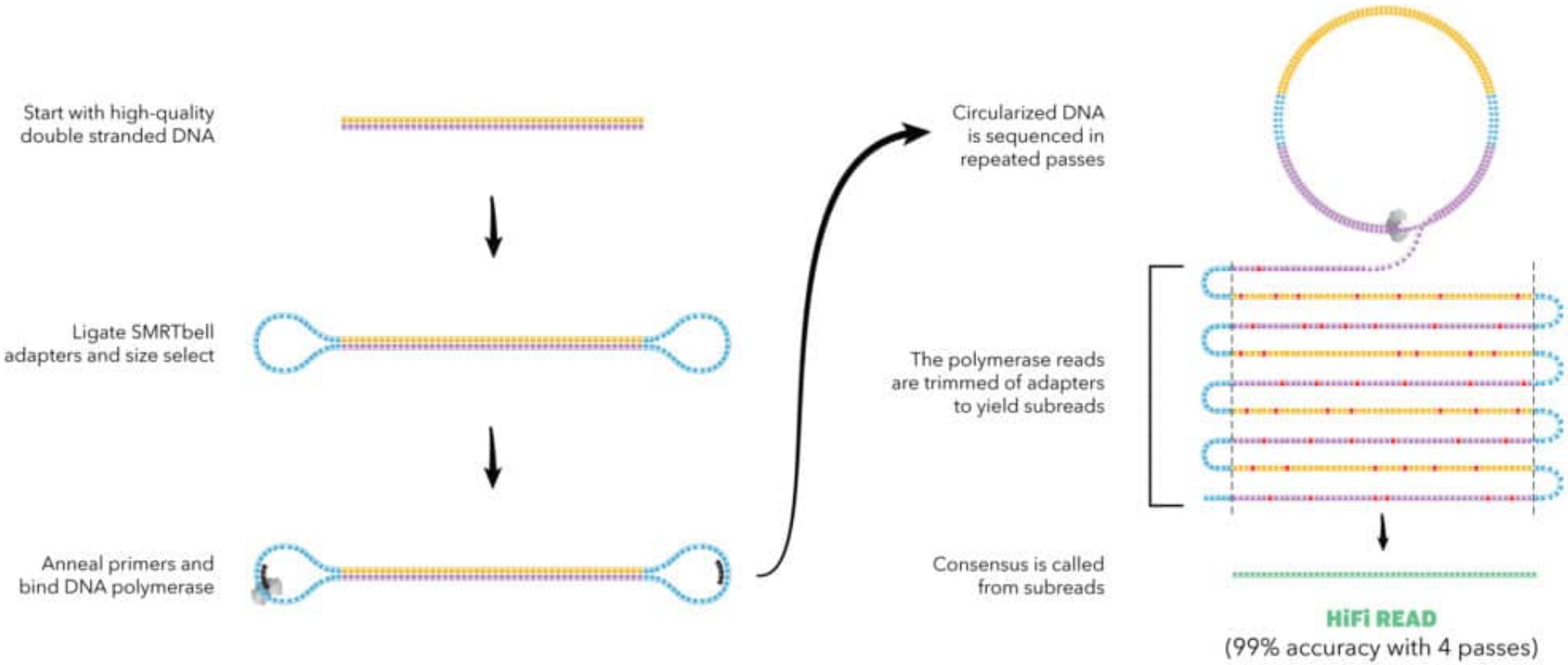
Subread error rate (single pass): 10-15%

This applies to the polymerase pass before circular consensus is formed—even on the Revio platform, although Revio primarily produces HiFi reads and doesn't typically output standalone subreads.

BAM ➜ FASTQ

BAM ➜ CCS.FASTX

Circular Consensus Sequences (CCS)



The CCS (Circular Consensus Sequencing) algorithm integrates multiple subreads to generate a high-accuracy consensus, but it has inherent resolution limits. Once the consensus quality reaches Q30–Q40 (99.9–99.99%), further accuracy gains plateau because the algorithm can no longer reliably distinguish true signal from low-probability noise. At this stage, most remaining errors stem from ambiguous or challenging regions—such as homopolymers—where the underlying signal is inherently difficult to interpret, regardless of how many passes are available.

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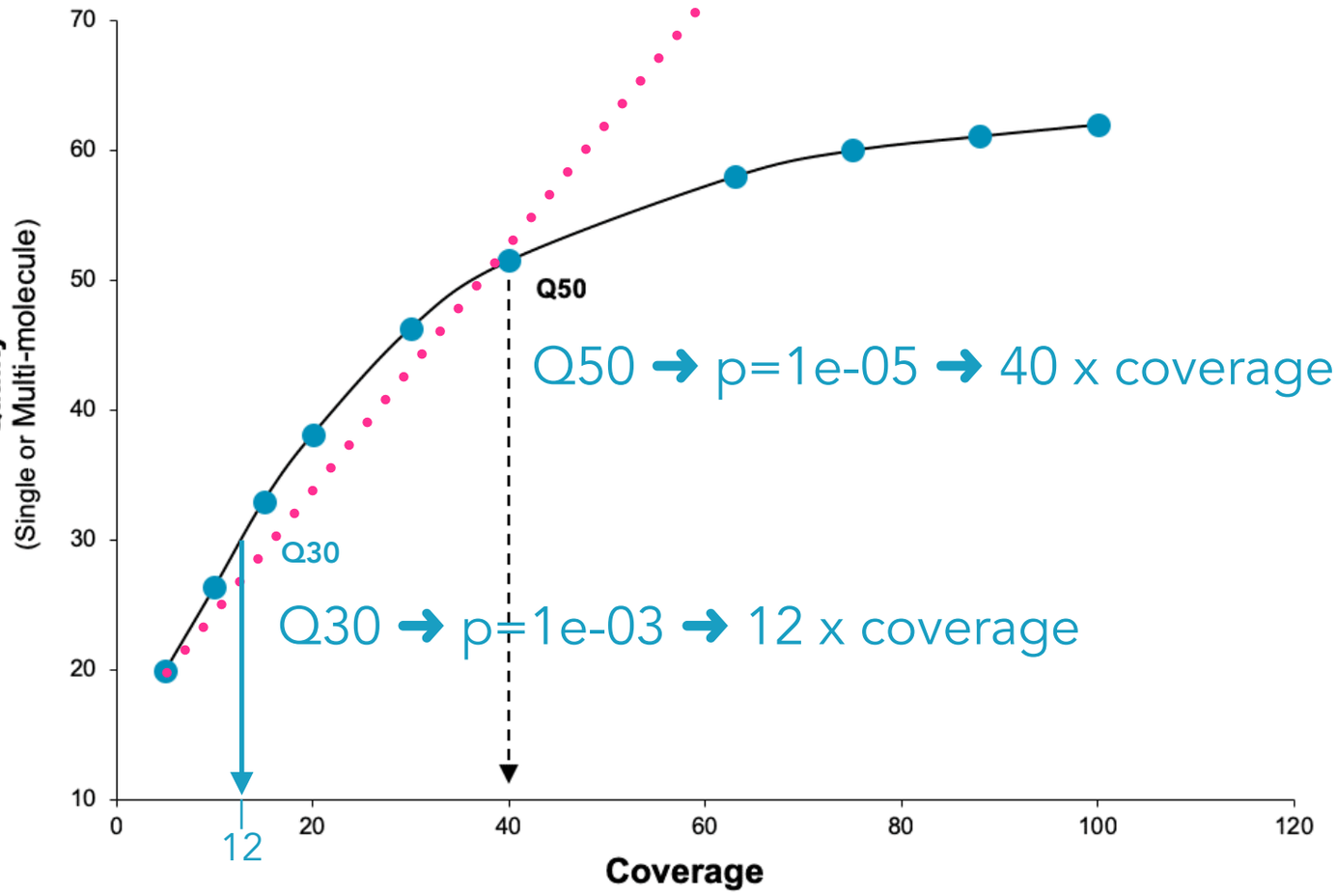
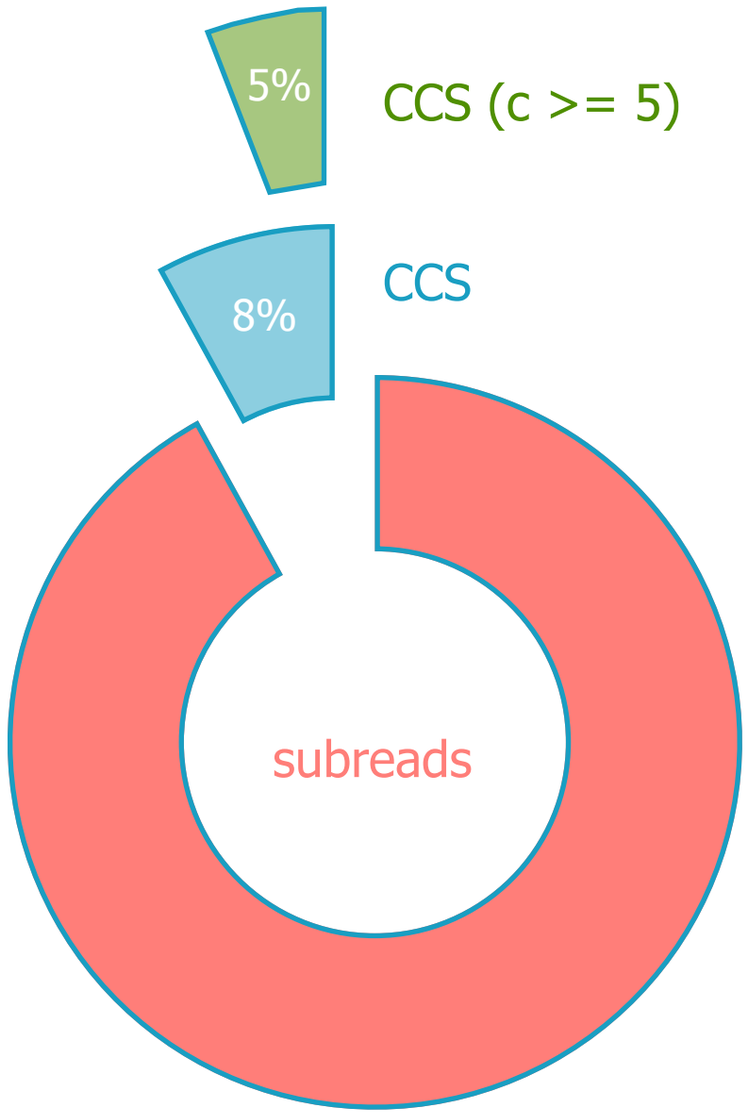
Why does it not improve anymore?



p: base-calling error probability

The plateau reflects a **point of optimal signal integration**—adding more data doesn't help if the error is already random and has been averaged out. It's a sweet spot where you get **maximum return on accuracy per pass** without wasting sequencing cycles.



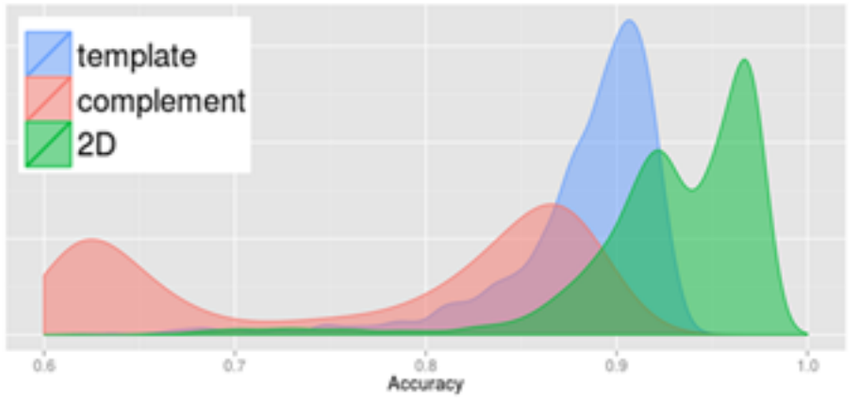
Subreads → CCS/HiFi conversion yield is usually **~5%** on **Sequel II** (e.g., 318 Gb → 16 Gb)

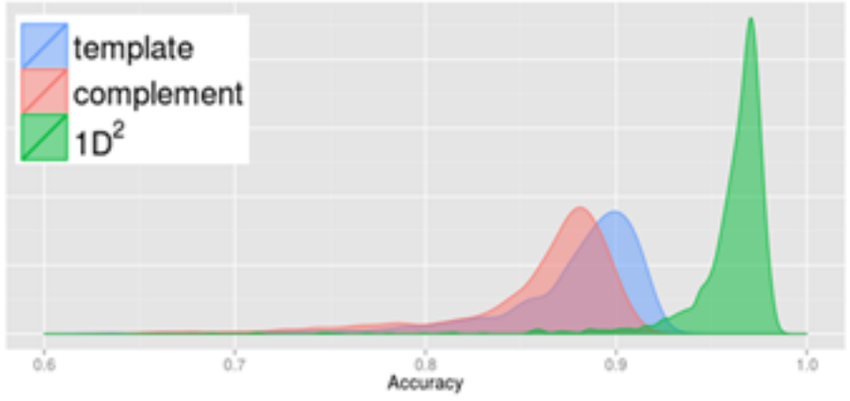
Zurich Genetic Diversity Centre

Revio raises CCS/HiFi yield ~3x (to 100–120 Gb per cell) while using the same subread base data.

```
# ZMWs input                      : 20,088,707
# ZMWs pass filters               : 14,389,230 (71.63%)
# ZMWs fail filters               :  5,699,477 (28.37%)
# - - - - - - - - - - - - - - - - : - - - - - - - - - - - -
# HiFi Reads                      : 13,057,507 (90.7%) <<<
# HiFi Read Length (mean, bp)     : 808
# HiFi Read Length (median, bp)   : 789
# HiFi Read Length N50 (bp)       : 789
# HiFi Read Quality (median)      : 81
# HiFi Number of Passes (mean)    : 49
#
# <Q20 Reads                      : 1,331,723
# <Q20 Yield (bp)                 : 1,057,777,942
# <Q20 Read Length (mean, bp)     : 794
# <Q20 Read Length (median, bp)   : 780
# <Q20 Read Quality (median)      : 17
#
# >=Q30 Reads                     : 11,403,584
# >=Q30 Yield (bp)                : 9,229,088,356
# >=Q30 Read Length (mean, bp)    : 809
# >=Q30 Read Length (median, bp): 790
# >=Q30 Read Quality (median)     : 92
```

Oxford NANOPORE Technologies





Read type	Mappable length (bp)				Error rate (Proportion of overall error) (%)			
	Mean	Median	Standard deviation	Maximum	Overall	Insertion	Deletion	Mismatch
PacBio CCS	1772	1464	1132	8006	1.72	0.087 (5.06)	0.34 (19.48)	1.30 (**75.46**)
PacBio subread	1570	1299	1076	16040	14.20	5.92 (**41.71**)	3.01 (21.17)	5.27 (37.12)
ONT 2D	1861	1754	882	9126	13.40	3.12 (23.30)	4.79 (35.70)	5.50 (**40.99**)
ONT 1D	1695	1602	824	9345	20.19	2.93 (14.51)	7.52 (37.24)	9.74 (**48.25**)

MITOCHONDRIAL DNA PART B: RESOURCES
2019, VOL. 4, NO. 1, 408–409
https://doi.org/10.1080/23802359.2018.1547133

Taylor & Francis
Taylor & Francis Group

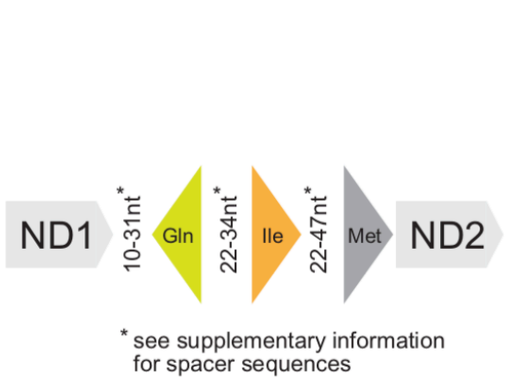
ARTICLE

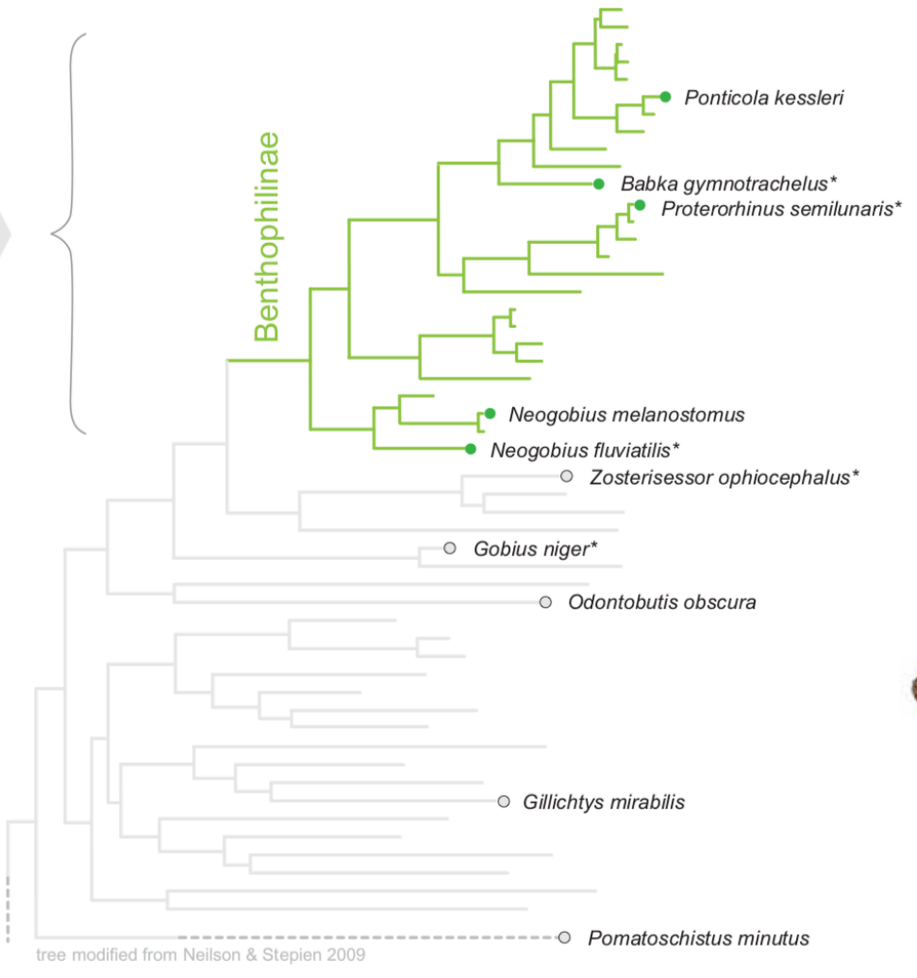
OPEN ACCESS Check for updates

Long-read sequencing of benthophilinae mitochondrial genomes reveals the origins of round goby mitogenome re-arrangements

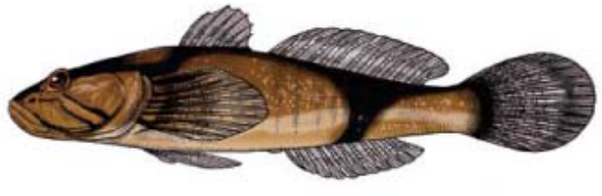
Silvia Gutnik[a], Jean-Claude Walser[b] and Irene Adrian-Kalchhauser[c]

[a]Biozentrum, Department Growth & Development, University of Basel, Basel, Switzerland; [b]Genetic Diversity Centre Zurich, ETH Zurich, Zurich, Switzerland;[c]Program Man-Society-Environment, Department of Environmental Sciences, University of Basel, Basel, Switzerland





Origin of the re-arranged **tRNA cluster** Gln, Ile, Met. Most Gobiidae carry the arrangement Ile, Gln, Met without spacers. Benthophilinae (subfamily of gobies) however carry the arrangement Gln, Ile, Met, and feature variable length spacers between the genes.



```
Illumina MiSeq R1 -> EE mean  0.1
Illumina MiSeq R2 -> EE mean  0.2
PacBio Sequel IIe -> EE mean  2.9
PacBio Revio      -> EE mean  1.2
ONT MinION        -> EE mean 30.7
```

Extras

Zurich Centre Genetic Diversity GDC

FastQC
(http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)

FASTX-Toolkit
(http://hannonlab.cshl.edu/fastx_toolkit/)

USEARCH
(https://www.drive5.com/usearch/)

PRINSEQ
(http://edwards.sdsu.edu/cgi-bin/prinseq/prinseq.cgi)

Galaxy
(http://galaxyproject.org)

Rqc
(https://bioconductor.org/packages/release/bioc/vignettes/Rqc/inst/doc/Rqc.html)

CLC Genomic Workbench
(http://www.clcbio.com/products/clc-genomics-workbench/)

Geneious
(http://www.geneious.com/)